

BIOREVERSIBLE DERIVATIVES OF 5-FLUOROURACIL (5FU):
IMPROVING DERMAL AND TRANSDERMAL DELIVERY
WITH PRODRUGS

BY

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KEY TO ABBREVIATIONS

bs	broad singlet
CDCl ₃	chloroform-d
(CD ₃) ₂ SO	dimethylsulfoxide-d ₆
CH ₃ CN	acetonitrile
d	doublet
dec	decomposition
dist t	distorted triplet
m	multiplet
q	quartet
R _f	retention factor
t	triplet

Abstract of Dissertation Presented to the Graduate School
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Fluorouracil (5FU) is an antineoplastic agent used topically for treatment of actinic keratoses, superficial basal cell carcinomas, psoriasis, and other skin conditions, but treatment is often ineffective due to its poor skin penetration properties. Four homologous series of bioreversible derivatives (prodrugs) of 5FU were synthesized, characterized, and their ability to penetrate through (transdermal) and accumulate in (dermal) the skin was evaluated. Six 1-alkyloxycarbonyl, six 1-acyl, four 1,3-bis-acyl, and four 3-acyl derivatives were studied.

Lipid solubility was a minimum of 40 times greater for the derivatives than for 5FU, and aqueous solubility was actually higher than 5FU for five derivatives. The

partitioning-based method for determining aqueous solubility gave reliable values for relative solubility in each homologous series, and overall, the values correlated well with conventionally determined solubilities. This was the first known successful attempt to obtain aqueous solubilities for chemically unstable prodrugs (e.g., 1-acyl and 1,3-bis-acyl derivatives).

Hydrolysis rates for N¹-substituents followed pseudo-first-order kinetics, but plots for N³-acyl hydrolysis were biexponential. An O²-acyl intermediate was proposed to account for the unusual hydrolysis and thermal decomposition of the N³-acyl group. X-ray crystal analysis showed that the N³-acyl group was oriented perpendicular to the 5FU ring and was sterically and electronically hindered from nucleophilic attack.

Skin penetration was 1.2 to nearly 40 times greater for the prodrugs than for 5FU. The highest flux was recorded for 1-acetyl-5FU, whereas 3-propionyl-5FU, 1-ethyloxycarbonyl-5FU, and 1,3-bis-acetyl-5FU exhibited the highest rates of delivery for their respective series. These derivatives were also the most aqueous soluble members of each series. This demonstrates the importance of biphasic solubility for achieving optimal transdermal delivery of 5FU from a homologous series of prodrugs.

Skin accumulation was highest for 1-acetyl-5FU and 1-propionyl-5FU (>18 times more than 5FU). Rapid hydrolysis of the 1-acyl series upon partitioning into the skin may

cause retention of highly polar 5FU in the more lipophilic regions of the epidermis.

Skin damage was only slightly increased with some prodrugs, all derivatives were stable in formulation, and overall, 1-acetyl-5FU was the best candidate for improving dermal or transdermal delivery of 5FU.

CHAPTER 1 INTRODUCTION

Fluorouracil

Fluorouracil (5FU) is one of the most widely used¹ and studied² anticancer agents. It is used in the palliative treatment of solid tumors and is most effective in combination with other antineoplastic agents³ and in conjunction with radiation therapy.² It has been used to treat carcinoma of the colon, rectum, breast, and stomach and, with less effectiveness, carcinoma of the ovary, cervix, urinary bladder, liver, and pancreas.⁴

Fluorouracil (5FU) is used as a single agent in topical preparations and is effective in treating actinic (solar) keratoses,⁵⁻⁸ superficial basal cell carcinomas,⁹⁻¹⁰ and psoriasis.¹¹⁻¹³ Topical 5FU has also been used to treat a variety of other precancerous conditions, malignant and benign tumors, and dermatoses.¹⁴ Commercial 5FU creams and solutions may be adequate for treating lesions on the face, but other areas, especially the forearms and hands, respond poorly probably due to lack of penetration by 5FU.¹⁵ Some conditions, such as psoriasis, respond to topical 5FU therapy when the drug is applied under an occlusive dressing.¹³

However, this is neither the most convenient nor comfortable treatment option.

The toxicity of 5FU is related to its effect on rapidly proliferating host cells especially those of the bone marrow and gastrointestinal lining. Toxicity following systemic therapy is common while adverse effects from topical therapy appear to be minimal, other than those associated with the local inflammatory reactions usually necessary for a therapeutic response.⁴

Fluorouracil Derivatives

Since 5FU was first synthesized¹⁶ and tested¹⁷ as an antitumor antimetabolite in 1957, numerous attempts have been made to improve its efficacy and reduce its toxicity. Derivatives of 5FU and its nucleosides, 5-fluorouridine (FUR) and 5-fluoro-2'-deoxyuridine (FdUR), have been developed with this in mind. The majority of these derivatives have been designed with the expectation that 5FU will be released in vivo, so they are essentially prodrugs.

A prodrug has been defined as "an agent which must undergo chemical or enzymatic transformation to the active or parent drug after administration, so that the metabolic product or parent drug can subsequently exhibit the desired pharmacological response" (p. 1275).¹⁸ Prodrugs, or bioreversible derivatives, of 5FU have included hydroxymethyl,¹⁹ alkyloxyalkyl,²⁰ acyloxyalkyl,²¹⁻²⁴ acyl,²⁵⁻²⁸

alkyloxycarbonyl,^{25,29-30} alkyloxycarbonyloxyalkyl,³¹⁻³³ alkyl-carbamoyl,³⁴⁻³⁷ aminoalkyl (Mannich base),³⁸⁻³⁹ phthalid-yl,⁴⁰⁻⁴¹ and derivatives containing sulfur rather than oxygen in some of the above functional groups.⁴²⁻⁴³ Polymer⁴⁴⁻⁴⁵ and peptide⁴⁶ derivatives have also been proposed as sources of 5FU in vivo. Currently, at least two bioreversible derivatives of 5FU, 1-(tetrahydrofuran-2-yl)-5-fluorouracil⁴⁷ and 1-hexylcarbamoyl-5-fluorouracil,⁴⁸ have been marketed in Japan.

The above is just a small sample of the synthetic literature involving 5FU. In addition, a large number of studies have been devoted to synthesizing analogs of FdUR and FdUR containing an N¹-glycosidic linkage. FdUR, or floxuridine, is available in the United States, but is only approved for intraarterial infusion.⁴ It appears to offer no advantage over intravenous 5FU, and this therapy is both hazardous and expensive.³ The nucleosides and their analogs are converted to 5FU in vivo by a pyrimidine phosphorylase. Since phosphorylase activity is higher in tumor tissue, it is believed that development of compounds that are good substrates for phosphorylase may lead to greater tumor specificity and decreased toxicity.⁴⁹

Fluorouracil Metabolism

Fluorouracil (5FU) undergoes extensive catabolic and anabolic metabolism. Approximately 15% of a single

intravenous dose of 5FU is excreted unchanged in the urine within six hours, and 90% of that is detected within the first hour.⁴ Approximately 80% of the dose is metabolized by the liver and extrahepatic tissues.¹ The principal catabolite of 5FU is α -fluoro- β -alanine (FBAL), which accounts for over 95% of the catabolic products found in the urine. Bile acid conjugates of FBAL represent the major biliary catabolites of 5FU.¹

A number of anabolic pathways have been characterized for 5FU, and one or more of them may be responsible for the activation and subsequent cytotoxicity of the drug. The most direct activation pathway utilizes orotidine monophosphate phosphoribosyl transferase (OMPT) to form 5-fluorouridine-5'-monophosphate (FUMP) from 5FU and 5-phosphoribosyl-1-pyrophosphate. FUMP can also be generated in two steps with uridine phosphorylase and uridine kinase. With two more kinases 5-fluorouridine-5'-triphosphate (FUTP) is formed which can be incorporated into ribonucleic acid (RNA) leading to RNA dysfunction.¹ Some cell lines appear to favor the OMPT pathway, while others favor the uridine phosphorylase pathway, and the FUTP formed from each is added to different fractions of RNA.⁵⁰

Some of the 5-fluorouridine-5'-diphosphate (FUDP) that is generated above as an intermediate can become a substrate for ribonucleotide reductase. The resulting deoxynucleotide, 5-fluoro-2'-deoxyuridine-5'-diphosphate (FdUDP), can form 5-fluoro-2'-deoxyuridine-5'-triphosphate (FdUTP) using

another kinase. At this point FdUTP can be incorporated into deoxyribonucleic acid (DNA), but the contribution to 5FU cytotoxicity by this mechanism is unclear. DNA protective enzymes, dUTPase and uracil-DNA glycosylase, which are responsible for keeping uracil residues out of DNA, target 5FU residues as well.⁵⁰

A diphosphohydrolase converts FdUTP to 5-fluoro-2'-deoxyuridine-5'-monophosphate (FdUMP). FdUMP can also be formed from 5FU by thymidine phosphorylase and thymidine kinase, although this may be quantitatively less important than other activation steps.¹ FdUMP binds covalently to thymidylate synthase and its cofactor, 5,10-methylene tetrahydrofolate, thereby preventing synthesis of thymidine-5'-monophosphate (dTMP) and subsequently, thymidine-5'-triphosphate (dTTP), an essential component in DNA synthesis. FdUMP has a greater affinity for thymidylate synthase than the normal substrate, 2'-deoxyuridine-5'-monophosphate (dUMP).¹

Either inhibition of thymidylate synthase by FdUMP or incorporation of FUTP into RNA can lead to cell death. The mechanism that is most important depends on the cell line being studied.⁵¹

Fluorouracil (5FU) may also affect glycoprotein and glycolipid metabolism, since it is known that FUDP sugars can be formed.⁵⁰ The possibility that membrane effects from altered glycoprotein synthesis could lead to cytotoxicity has been noted, but has not been well studied.¹

Structure of the Skin

Fluorouracil (5FU) is typical of polar, high-melting, heterocyclic compounds which exhibit poor skin permeability.³⁸ Since the goal of this project is to improve dermal and transdermal delivery of 5FU, it is necessary to examine the structure of the barrier to be penetrated, the skin.

The skin is composed of two major tissue layers, the epidermis and dermis. The epidermis is a continuous, elastic sheet that is interrupted only by glandular pores and hair follicles. It consists of four definable sublayers and averages 75 to 150 μm in thickness. The basal cell layer, which borders the dermis, is a single layer of keratinocytes. This is the germinal layer, and all epidermal cells are initially formed here before moving outward to the next layer, the stratum spinosum. The basal cells are cuboidal or columnar in shape, while the stratum spinosum cells are polyhedral. By the time the cells have migrated to the next layer, the stratum granulosum, they have become flattened and contain characteristic keratohyalin granules. The stratum granulosum marks the transition between nucleated cells and the anucleated stratum corneum. The stratum corneum cells in the last layer are highly keratinized, markedly flattened cells in which cellular components, such as mitochondria and ribosomes, have degraded along with the nucleus. Stratum corneum normally consists of about 15 to 20 layers, but each

layer is only about 0.5 μm in thickness. The outer layers are continuously desquamated and replaced from below. The entire transit time from basal layer to desquamation is 26 to 42 days. The cells of the epidermis are attached to each other by desmosomes, which degrade just prior to desquamation.⁵²

Below the epidermis lies the dermis which constitutes the bulk of the approximately 2 mm thickness of human skin. The two layers are anchored to the basal lamina by various fibrils and microfibrils. The attachment is enhanced by the interlocking nature of the junction.⁵²

The dermis consists of two regions, the papillary dermis and reticular dermis. The papillary dermis is the thinner, outermost region that is molded against the ridges and grooves of the epidermis. It contains small, loosely distributed fibrils and encloses the microcirculatory blood and lymph vessels. In contrast, the collagen bundles and elastin fibers of the reticular dermis are more densely packed, and this region is relatively acellular and avascular. Collagen is the major component of the dermis, and it gives the skin its tensile strength. The dermis also contains nerves, excretory and secretory glands, and hair follicles.⁵²

Mechanisms of Transdermal Penetration

For most substances, the stratum corneum provides the rate-limiting barrier to skin penetration.⁵³⁻⁵⁵ The stratum corneum is 75-85% protein (dry weight),⁵⁴ mostly intracellular keratin, while the intercellular space is a lipid-enriched region. During the outward migration of the keratinocytes through the epidermis, lamellar bodies are synthesized, which contain lipids, polysaccharides, and hydrolytic enzymes. As the granular cells prepare to enter the stratum corneum, the lamellar bodies move to the cell periphery and empty their contents into the spaces between the cells.⁵⁶ The corneocytes and intercellular lipids are arranged in a brick and mortar fashion,⁵⁷ and the result is a very dense (1.4 g/cm^3 in the dry state),⁵⁸ highly efficient moisture barrier.

In order for a substance to penetrate the stratum corneum, it must either (1) cross through the densely packed corneocytes, (2) traverse through the intercellular region, thereby avoiding the keratinized cells, or (3) bypass the stratum corneum completely by diffusing through shunt pathways such as sweat ducts and hair follicles.⁵³ The shunt pathways are not considered to be significant, especially when steady state diffusion has been attained,⁵⁹ and these pathways are generally disregarded in discussions of penetration mechanisms.

Until recently, it was thought that the transcellular pathway was the primary route through the stratum corneum. This was based, at least in part, on a gross underestimation of the volume of the intercellular space.⁶⁰ The current belief is that passive diffusion occurs predominantly through the intercellular channels.⁶¹⁻⁶² While this seems logical for lipophilic compounds, this pathway has also been shown to be the dominant route for mercuric chloride, an ionic compound. Boddé et al.⁶³ showed that mercuric sulfide could be precipitated in the stratum corneum after topical application of mercuric chloride. They found that mercuric cation accumulates initially in the intercellular spaces throughout the stratum corneum. Intracellular uptake was observed later, but only in the apical corneocytes by way of disintegrating desmosomal attachments. Since the intercellular lipids are arranged in multiple lamellar bilayers,⁶⁴ the mercuric cation, and possibly other ions and hydrophilic molecules, may diffuse through the interlamellar, hydrophilic channels that are associated with the polar headgroups of the lipids.⁶³

While most of the current research is directed toward understanding the intercellular region, Barry⁵⁹ cautions that the transcellular route should not be dismissed as unimportant, especially in penetration enhancer research.

Passive Diffusion

Although the skin is not a homogeneous tissue, it has the characteristics of a passive diffusion barrier. Fick's first law of diffusion defines flux as the amount of material flowing through a unit cross section of a barrier in a unit time.⁶⁵ Flux is also proportional to the concentration gradient, and the first law can be written:

$$J = -D \cdot dC/dx \quad (1)$$

where J is the flux, D is the diffusion coefficient of the penetrant in the barrier, and dC/dx is the concentration gradient. While the first law is used to describe steady-state diffusion, Fick's second law describes the change in concentration with respect to time at a specific location.⁶⁵ Our interest is in steady-state diffusion, and the second law will not be discussed further.

The concentration gradient term (dC/dx) from Fick's first law can be approximated by $(C_1 - C_2)/h$, where C_1 is the concentration in the barrier on the donor side of the barrier, while C_2 is the concentration in the barrier on the receptor side, and h is the thickness of the diffusional barrier. Therefore, the expression becomes

$$J = D \cdot (C_1 - C_2) / h \quad (2)$$

For skin penetration studies, "sink" conditions can be assumed which means that $C_2 = 0$, and

$$J = D \cdot C_1 / h \quad (3)$$

Since C_1 is essentially the concentration in the first layer(s) of skin, C_1 will now be referred to as C_s , so

$$J = D \cdot C_s / h \quad (4)$$

While C_s is not usually a known quantity, it can be represented instead by the product of the penetrant concentration in the donor vehicle (C_v) and the membrane-vehicle partition coefficient ($K_m = C_s / C_v$), and the expression now becomes

$$J = D \cdot K_m \cdot C_v / h \quad (5)$$

This expanded form of Fick's first law is often cited and has been verified by data from numerous skin penetration studies.⁶⁶

Another useful measure of skin penetration is the permeability coefficient (P). This is simply the concentration-normalized flux which is expressed:

$$P = J / C_v = D \cdot K_m / h \quad (6)$$

Permeability coefficients are often used when comparing a single penetrant in a series of vehicles or when studying an homologous series of penetrants.

Enhancement of Skin Penetration

The driving force for skin penetration is the membrane-vehicle partition coefficient (K_m). If a penetrant is delivered in a saturated solution in the presence of excess solid, then the chemical potential, or escaping tendency, is maximized, and the actual concentration in the vehicle (C_v)

is irrelevant. Since the membrane thickness (h) and diffusion coefficient (D) remain relatively constant (D is inversely proportional to the cube root of the molar volume according to the Stokes-Einstein equation), K_m is the only variable that can substantially influence the flux.

There are several general approaches for enhancing skin penetration. Four of these--occlusion, use of penetration enhancers, iontophoresis, and sonophoresis--produce their enhancement effects by changing the barrier properties of the skin. Occlusion involves covering the application site, impeding transepidermal water loss, and increasing the hydration state of the skin.⁶⁷ With penetration enhancers, accelerants, or promoters in topical formulations, the reversible reduction of barrier resistance in the stratum corneum is the goal, and ideally, incorporation of the enhancer into the skin will not result in cell damage.⁵⁹ Iontophoresis is a technique in which electroosmotic volume flow from an applied electric field leads to increases in mass transfer in excess of passive diffusion.⁶⁸ Sonophoresis uses ultrasonic frequencies to increase skin penetration.

The other two methods, formulation (without penetration enhancers) and the use of prodrugs, do not disrupt the barrier layer. Essentially, the formulation approach involves changing the penetrants solubility in the vehicle by changing the vehicle. The effect on flux of this approach is indeterminate according to equation (5). If the solubility in the vehicle (C_v) is increased, then the partition

coefficient (K_m) decreases and vice versa. In a series of papers,⁶⁹⁻⁷² Sloan and coworkers suggested that a parabolic relationship exists for $\log K_m$ and $\log P$ when they are plotted against vehicle polarity. $\log K_m$ and $\log P$ reach a minimum where vehicle polarity is equal to penetrant polarity or in other words, where penetrant solubility in the vehicle is the greatest. While this relationship might be expected, a more interesting finding was that in most instances fluxes are also lower from those vehicles in which the penetrants are most soluble. While a formulation approach may permit some improvement in skin penetration values, the maximum achievable levels appear to be limited.

The final method for enhancing skin penetration is the prodrug approach. This is the only method in which a substantial increase in K_m can be realized, and according to equation (5), this should translate into substantially improved skin penetration. It was this knowledge that led to adoption of the prodrug method for the current project.

Prodrugs and Skin Penetration

The term, prodrug, was defined earlier in this chapter. Prodrugs, bioreversible derivatives, or latentiated drugs are simply compounds which undergo biotransformation before producing their pharmacological effects.⁷³

Generally, prodrugs are designed to overcome some kind of barrier to a drug's usefulness. These barriers can

include (1) premature metabolism prior to reaching the active site, (2) too rapid absorption and distribution when prolonged action is needed, (3) toxicity associated with (a) local irritation or (b) distribution to tissues other than the target site, (4) poor site specificity leading to subtherapeutic levels at the target site, and (5) generally poor physical chemical properties resulting in (a) solubility problems in the dosage form or (b) poor absorption across biological membranes such as the blood brain barrier, gastrointestinal lining, or skin.⁷⁴ Obviously, it is the latter problem that is addressed in the current project.

The idea that improving skin penetration is a solubility problem has been documented in this chapter, and the importance of biphasic (lipid and aqueous) solubility is well recognized.^{66,75-76} In a recent review of prodrug approaches for improving dermal delivery, numerous literature examples are cited in which both solubility and skin penetration data are presented.⁷⁷ The evidence strongly suggests that:

although an increase in lipid solubility due to transient masking of a polar functional group almost always results in enhanced dermal delivery of the parent drug, in order to optimize delivery, it is necessary to use the members of the (homologous) series (of prodrugs) that are more water soluble than the parent drug or that are the more water-soluble member(s) of the series (p. 68).⁷⁷

Derivatization to improve solubility characteristics of polar heterocycles with amide and/or imide functional groups is relatively easy to accomplish. For example, successive methylation of uracil at the N¹- and N³-positions, while

obviously improving lipid solubility, also increases water solubility from 3 mg/mL for uracil to 500 mg/mL for 1,3-di-methyluracil even though the methyl group is hydrophobic itself. Melting points are also decreased as the two intermolecular hydrogen-bonding N-H sites are masked.⁷⁸ Since alkyl groups such as methyl are stable and therefore not bioreversible, they are not candidates to function as prodrug promoieties. However, many other derivatives, such as those cited earlier, when linked to the N¹- or N³-site of 5FU, do qualify as promoieties and could potentially give 5FU the improved solubility characteristics necessary for enhanced skin penetration.

Of the many studies devoted to making bioreversible derivatives of 5FU, only a few have been directed at improving its topical delivery. Mollgaard et al.²¹ looked at two 1-acyloxymethyl derivatives of 5FU. One of the compounds delivered 5FU more readily than 5FU itself through excised human skin. Both compounds showed greatly increased lipid solubilities with only slightly reduced water solubilities. Hydrolysis of these derivatives was attributed to cutaneous metabolism by hydrolytic enzymes.

Three 1,3-bis-aminomethyl (Mannich base) derivatives of 5FU were prepared by Sloan and coworkers,³⁸⁻³⁹ and their topical delivery was studied using hairless mouse skin. Solubilities in lipid and aqueous phases were substantially increased as were the rates of delivery through the skin of 5FU from these prodrugs. Due to the instability of these

compounds in water, no enzymatic activation was necessary to release the parent compound. In a later report,⁷⁹ one of these prodrugs was compared with a number of 5FU formulations including four commercially available creams and solutions. The prodrug outperformed the formulations by a minimum of four times in terms of 5FU delivered through hairless mouse skin.

Sasaki et al.³⁷ studied the delivery of 1-alkylcarbamoyl derivatives of 5FU through rat skin. All three derivatives (butyl, hexyl, and octyl) were more effective in delivering 5FU than 5FU itself. The lipid solubilities of the three derivatives were comparable with each other and much higher than 5FU. The aqueous solubilities, while less than 5FU, showed an order of magnitude decrease between each derivative beginning with the butyl derivative. Interestingly, the best performing compound was the least lipid-soluble and most water-soluble derivative, the butyl derivative.

Dermal versus Transdermal Delivery

It is important to make a distinction between dermal and transdermal delivery. Most in vitro skin penetration testing is done with excised skin to which a drug in a formulation is applied on the donor side and samples are removed from the receptor side. These experiments give information on transdermal rates of delivery.

Topical 5FU works on afflicted cells in the epidermal region of the skin. This is considered dermal delivery. Transdermal techniques provide valuable information for dermally targeted drugs since they indicate how effectively the drugs penetrate the barrier layer of the skin. Other experiments can be done to augment the transdermal results such as measuring accumulation of the drug in the skin.

The correlation between transdermal delivery rates and epidermal uptake was studied by Sloan et al.⁷⁹⁻⁸⁰ Incorporation of ³H-deoxyuridine into epidermal DNA of live hairless mice was quantitated by scintillation counting following application of various 5FU formulations and a 5FU prodrug. The prodrug, which had the highest in vitro transdermal flux, was also the most effective at inhibiting epidermal DNA synthesis in vivo. The correlation was also good among the formulations.

Cutaneous Metabolism

When a prodrug is applied topically and targeted for a dermal site, it is essential that the parent drug is released before the prodrug leaves the epidermal region. If a prodrug has a relatively short half-life under physiological conditions, such as the aforementioned Mannich-base prodrugs of 5FU, then release of the parent drug will probably occur either prior to or during its transit through the viable epidermis. However, if a compound depends on enzymatic

rather than chemical activation, then an appropriate cutaneous enzyme must be present.

The ability of the skin to metabolize drugs and other foreign compounds is well known.⁸¹⁻⁸² Phase I reactions (oxidation, reduction, and hydrolysis) and phase II conjugations are known to occur. Drug-metabolizing enzymes are distributed in all layers of the skin and the appendages.

Of particular interest in prodrug chemistry is the presence of nonspecific esterases in the skin.⁸² The predictable metabolism of esters by these hydrolytic enzymes makes this functional group a popular choice for prodrug synthesis.

Hairless Mouse Skin as a Model Membrane

A number of animal skins have been suggested as model membranes for skin penetration studies. The two most common models for in vitro diffusion studies are human and hairless mouse skin. While the advantages of human skin are obvious, there are also disadvantages. Human skin can be difficult to obtain and store,⁸³ it can be expensive,⁸³ and it is known to have high barrier variability.⁸³⁻⁸⁴ Factors such as age, diet, and disease state may not be well controlled with human skin.⁸⁵ Hairless mouse skin, on the other hand, is easily obtained and prepared, and other factors can ordinarily be controlled.

The importance of hairless mouse skin for in vitro experimentation has been noted⁸⁶ despite its generally greater permeability when compared to human skin. It has been suggested that this difference may actually be an asset in that small changes in permeability will be amplified in the mouse skin model.⁸⁶

A major criticism of hairless mouse skin involves its ability to withstand the effects of hydration,⁸⁷⁻⁸⁸ a necessary condition for controlled in vitro diffusion experiments. Permeability increases as a function of hydration time have been attributed to breakdown of the stratum corneum barrier in these studies. A recent report,⁸⁹ however, suggested that the absence of an adequate preservative in the receptor phase may actually be responsible for breakdown of the skin. Skin penetration data was collected for delivery of theophylline from a propylene glycol vehicle following skin hydration periods ranging from 4 to 120 hours. It was found that increased theophylline flux and loss of barrier function corresponded to the presence of microbial growth in the receptor phase. When microbial growth was completely inhibited, fluxes were essentially constant for all hydration periods.⁸⁹ Finally, with regard to hydration, Scheuplein and Ross noted that "even well-hydrated stratum corneum preferentially dissolves lipid-soluble molecules, so that the selective permeability of these molecules is preserved" (p. 353).⁹⁰

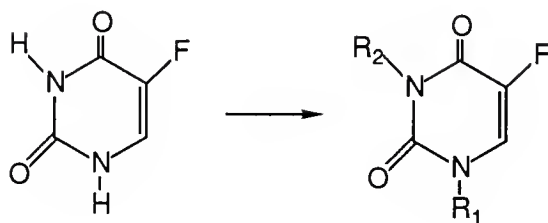
Another difference between human and hairless mouse skin could be significant in prodrug design. Rat and mouse skins apparently have higher levels of enzymatic activity for drug metabolism than human skin. Esterase activity, specifically, appears to be low in human skin.⁵⁴

Research Proposal

The overall goal of the present research is to develop prodrug derivatives of 5FU with solubility characteristics and skin penetration properties superior to 5FU itself. The derivatives should be stable in formulation, but should readily convert to 5FU in the skin since the purpose of topical 5FU therapy is to deliver 5FU dermally, not transdermally. In pursuit of this goal, it is hoped that experimental data will be obtained which supports a solubility-based method for designing prodrugs with optimized topical delivery characteristics. The objectives for meeting this goal are to:

- 1) synthesize several homologous series of acylated derivatives of 5FU,
- 2) verify structures by melting point, elemental analysis (novel compounds), and standard spectral techniques,
- 3) determine physical chemical properties such as lipid and aqueous solubilities, partition coefficients, and rates of hydrolysis, and
- 4) determine skin penetration parameters and quantitate skin accumulation using hairless mouse skin as the model membrane.

Table 1-1. Structures of 5FU and prodrug derivatives of 5FU.



Series	R ₁	R ₂
1-alkyloxycarbonyl-5FU (I)	-(C=O)O(CH ₂) _n CH ₃	-H
1-acyl-5FU (II)	-(C=O)(CH ₂) _n CH ₃	-H
1,3-bis-acyl-5FU (III)	-(C=O)(CH ₂) _n CH ₃	-(C=O)(CH ₂) _n CH ₃
3-acyl-5FU (IV)	-H	-(C=O)(CH ₂) _n CH ₃

Four series of compounds have been selected as potential prodrugs of 5FU. They are the 1-alkyloxycarbonyl (I), 1-acyl (II), 1,3-bis-acyl (III), and 3-acyl (IV) derivatives of 5FU (Table 1-1). Fluorouracil (5FU) has two acidic pKa values, 8.0 and 13.0.⁹¹ Spectral studies have shown that the monoanion is actually a mixture of N¹- and N³-anions.³⁰ A comparison of ionization constants for 5FU derivatives that are identically substituted at the N¹- or N³-positions^{22,30} suggests that the N³-position is probably the most acidic. However, the N¹-position is the most reactive site for both synthesis and hydrolysis of acylated derivatives of 5FU.

The three series of acyl derivatives were chosen for two reasons; they exhibit high lipid and aqueous solubilities, and they readily hydrolyze without enzymatic activation.²⁶ While rapid hydrolysis is seen as a drawback by some investigators,^{26,42} it may actually be an advantage when developing prodrugs for dermal delivery.^{38-39,92} The 1-alkyloxycarbonyl series was selected for comparison with the 1-acyl series since it shows good chemical stability, but rapid enzymatic hydrolysis.³⁰ Various members of each series have been synthesized previously,^{25-27,30,93} but homologous series have not been examined, and nobody has studied their applicability for dermal delivery.

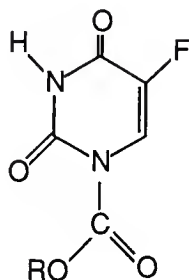
CHAPTER 2 1-ALKYLOXYCARBONYL DERIVATIVES

Introduction

Alkyloxycarbonyl derivatives of 5-fluorouracil (5FU) have previously been studied as potential sources of 5FU in vivo.^{25,29-30} When substitution is at the N³-position of 5FU, the derivatives are chemically stable. Their hydrolyses in human plasma and liver homogenate are also slow enough to raise questions about their usefulness as prodrugs for the oral or rectal delivery of 5FU.²⁹ Thus, there is no doubt that they are too stable to serve as prodrugs for dermal delivery. On the other hand, substitution at the N¹-position produces compounds that are relatively stable chemically,³⁰ but which are sufficiently labile in the presence of enzymes³⁰ to justify consideration as dermal prodrugs.

Six straight-chain 1-alkyloxycarbonyl derivatives were selected for study. The derivatives and their structures are shown in Table 2-1.

Table 2-1. Structures of 1-alkyloxycarbonyl derivatives.



Compound	R
1-methyloxycarbonyl-5FU (1)	-CH ₃
1-ethyloxycarbonyl-5FU (2)	-CH ₂ CH ₃
1-propyloxycarbonyl-5FU (3)	-(CH ₂) ₂ CH ₃
1-butyloxycarbonyl-5FU (4)	-(CH ₂) ₃ CH ₃
1-hexyloxycarbonyl-5FU (5)	-(CH ₂) ₅ CH ₃
1-octyloxycarbonyl-5FU (6)	-(CH ₂) ₇ CH ₃

Materials and Methods

Synthesis

Melting points (mp) were determined with a Thomas-Hoover capillary melting point apparatus and are uncorrected. Elemental microanalyses were obtained for all novel compounds through Atlantic Microlab, Incorporated in Norcross, Georgia. Proton nuclear magnetic resonance (^1H NMR) spectra were obtained at 90 MHz on a Varian EM-390 spectrometer. Chemical shifts (δ) are reported in parts per million (ppm) from the internal standard, tetramethylsilane (TMS). Coupling constants (J) are expressed in cycles per second (Hz). Infrared (IR) spectra were recorded with a Perkin-Elmer 1420 spectrophotometer and absorbances are reported in cm^{-1} . Ultraviolet (UV) spectra were obtained with a Cary 210 or Shimadzu UV-265 spectrophotometer. Maximum absorbances are reported in nm along with the molar absorptivities (ϵ) in L/mol.

1-Alkyloxycarbonyl-5-fluorouracil (general procedure)

To 0.66 g (0.01 mol) of 85% potassium hydroxide dissolved in methanol (20-50 mL) was added 1.33 g of 5FU (0.0101 mol). Slightly more than an equivalent of 5FU was used to prevent excess base from catalyzing the aldol condensation of acetone. When present, condensation products complicated product isolation. The methanol suspension was

stirred for 30 minutes, and the methanol was evaporated under reduced pressure. The potassium salt was suspended in acetone (25-50 mL) which was evaporated under reduced pressure to remove residual methanol. The salt was resuspended in acetone (25-50 mL), and the suspension was added dropwise over a three minute period to a well stirred acetone (20 mL) solution containing 1.0 to 1.2 equivalents of the appropriate alkyl chloroformate. The mixture was stirred at room temperature for 60 minutes. The mixture was filtered, and the residue was washed with acetone (20 mL). The combined acetone solutions were evaporated under reduced pressure, and the solid residue was crystallized from an appropriate solvent or solvent combination.¹

1-Methyloxycarbonyl-5-fluorouracil (1)

Crystallization from acetone gave 1.36 g of **1** (72%): mp 158-60 °C (lit.³⁰ mp 159-60 °C); IR (KBr) 1695, 1710, 1740, and 1760 cm⁻¹ (C=O); ¹H NMR [(CD₃)₂SO] δ 3.86 (s, 3H, CH₃) and 8.16 (d, J=7 Hz, 1H, C⁶-H); UV_{max} (CH₃CN) 254 nm (ε=9.63×10³).

1-Ethyloxycarbonyl-5-fluorouracil (2)

Crystallization from acetone/ether gave 1.31 g of **2** (65%): mp 127-8 °C (lit.³⁰ mp 126-8 °C); IR (KBr) 1690, 1730, and 1750 cm⁻¹ (C=O); ¹H NMR [(CD₃)₂SO] δ 1.31 (t, J=7 Hz, 3H, CH₃), 4.31 (q, J=7 Hz, 2H, OCH₂), and 8.16 (d, J=7 Hz, 1H, C⁶-H); UV_{max} (CH₃CN) 254 nm (ε=9.86×10³).

¹Several compounds in this series were provided by Kenneth B. Sloan, Ph. D. based on the author's procedure.

1-Propyloxycarbonyl-5-fluorouracil (3)

Crystallization from acetone/ether gave 1.37 g of **3** (64%): mp 124-6 °C; IR (KBr) 1690, 1730, and 1755 cm^{-1} (C=O); ^1H NMR [$(\text{CD}_3)_2\text{SO}$] δ 0.95 (t, $J=7$ Hz, 3H, CH_3), 1.70 (m, 2H, OCH_2CH_2), 4.23 (t, $J=7$ Hz, 2H, OCH_2), and 8.15 (d, $J=7$ Hz, 1H, $\text{C}^6\text{-H}$); UV_{max} (CH_3CN) 254 nm ($\epsilon=1.001 \times 10^4$).

Anal. Calc. for $\text{C}_8\text{H}_9\text{FN}_2\text{O}_4$: C, 44.45; H, 4.20; N, 12.96.
Found: C, 44.53; H, 4.23; N, 12.89.

1-Butyloxycarbonyl-5-fluorouracil (4)

Crystallization from dichloromethane/hexane gave 1.33 g of **4** (58%): mp 97-8 °C (lit.³⁰ mp 102-4 °C); IR (KBr) 1695, 1735, and 1765 cm^{-1} (C=O); ^1H NMR [$(\text{CD}_3)_2\text{SO}$] δ 0.91 (t, $J=7$ Hz, 3H, CH_3), 1.3-1.8 (m, 4H, $\text{OCH}_2\text{CH}_2\text{CH}_2$), 4.27 (t, $J=6$ Hz, 2H, OCH_2), and 8.13 (d, $J=7$ Hz, 1H, $\text{C}^6\text{-H}$); UV_{max} (CH_3CN) 254 nm ($\epsilon=9.93 \times 10^3$).

1-Hexyloxycarbonyl-5-fluorouracil (5)

Crystallization from dichloromethane/hexane gave 1.27 g of **5** (49%): mp 66-7 °C (lit.⁹³ mp 68-9 °C); IR (KBr) 1690, 1730, and 1750 cm^{-1} (C=O); ^1H NMR [$(\text{CD}_3)_2\text{SO}$] δ 0.87 (distd t, 3H, CH_3), 1.1-1.8 (m, 8H, $\text{OCH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2$), 4.26 (t, $J=6$ Hz, 2H, OCH_2), and 8.13 (d, $J=7$ Hz, 1H, $\text{C}^6\text{-H}$); UV_{max} (CH_3CN) 254 nm ($\epsilon=1.004 \times 10^4$).

1-Octyloxycarbonyl-5-fluorouracil (6)

Crystallization from dichloromethane/hexane gave 1.80 g of **6** (61%): mp 97-8 °C; IR (KBr) 1690, 1730, and 1750 cm^{-1} (C=O); ^1H NMR [$(\text{CD}_3)_2\text{SO}$] δ 0.87 (distd t, 3H, CH_3), 1.2-1.9

(m, 12H, $\text{OCH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2$), 4.27 (t, $J=6$ Hz, 2H, OCH_2), and 8.15 (d, $J=7$ Hz, 1H, $\text{C}^6\text{-H}$); UV_{max} (CH_3CN) 254 nm ($\epsilon=1.009 \times 10^4$).

Anal. Calc. for $\text{C}_{13}\text{H}_{19}\text{FN}_2\text{O}_4$: C, 54.53; H, 6.69; N, 9.79. Found: C, 54.46; H, 6.73; N, 9.77.

Lipid Solubility

Lipid solubilities were determined using isopropyl myristate (IPM), a commercial vehicle used in cosmetics and topical medicinals,⁹⁴ as the lipid solvent. The use of IPM as a model lipophilic vehicle in skin penetration studies is well established.^{77,95}

Three suspensions of each derivative were stirred at 22 ± 1 °C for 48 hours. The suspensions were filtered through 0.45 μm nylon filters, and the saturated solutions were diluted in acetonitrile and analyzed by UV spectroscopy. Solubilities were calculated using Beer's Law:

$$A = \epsilon \cdot C \cdot d \quad (1)$$

where A is the absorbance, ϵ is the molar absorptivity, C is the concentration in mol/L, and d is the path length of the cuvette in cm. Molar absorptivities were predetermined in triplicate in acetonitrile at 254 nm.

Aqueous Solubility

For direct measurement of aqueous solubilities, three suspensions of each derivative were vigorously stirred in

0.05 M acetate buffer (pH=4.0) at 22 ± 1 °C for 60 minutes. The suspensions were filtered through 0.45 μ m nylon filters, and the saturated solutions were diluted in acetonitrile and analyzed by UV spectroscopy. Solubilities were calculated using Beer's Law as previously described.

Partition Coefficients

The partitioning-based method for determining aqueous solubility utilized the saturated IPM solutions from the lipid solubility study. For most compounds, equal volumes (1 mL) of saturated IPM solution and 0.05 M acetate buffer (pH=4.0) were used. The use of equal or near-equal phase volumes is known to facilitate rapid equilibrium.⁹⁶ The two phases were mixed thoroughly for ten seconds and allowed to separate for 60 seconds. A preliminary study showed that there was virtually no difference in partition coefficient (PC) values when partitioning was carried out for 10, 20, or 30 seconds (see Chapter 3). The IPM layers were diluted in acetonitrile and analyzed by UV spectroscopy. The IPM-buffer partition coefficients were calculated as follows:

$$PC = A_{\text{after}} / (A_{\text{before}} - A_{\text{after}}) \cdot V_{\text{AQ}} / V_{\text{IPM}} \quad (2)$$

where A_{after} is the absorbance from the IPM layer after partitioning, A_{before} is the absorbance from the IPM layer before partitioning, V_{AQ} is the volume of the aqueous phase, and V_{IPM} is the volume of the IPM phase. Estimated aqueous

solubilities (S_{AQ}) were calculated from the IPM solubility (S_{IPM}) and the partition coefficient:

$$S_{AQ} = S_{IPM}/PC \quad (3)$$

Partitioning was carried out in triplicate for a fixed volume ratio for each derivative. For those compounds with large differences in solubility in one phase relative to the other, volume ratios (IPM:buffer) other than 1:1 were necessary, but the ratio never exceeded 10:1 or 1:10.

Hydrolysis Kinetics

Hydrolysis rates have previously been reported for several members of this homologous series.^{30,93} In the present study, hydrolysis rates were determined at 32 °C for 1-methyloxycarbonyl-5FU (1) in 0.05 M phosphate buffer (pH=7.1, I=0.12) and in the same buffer with 0.11% formaldehyde (3.6×10^{-2} M). The rate in the presence of formaldehyde was determined for comparison with the rate in plain buffer since formaldehyde was used as a preservative in the diffusion cell experiments described in the following section.

The hydrolyses were followed by high performance liquid chromatography (HPLC). The HPLC system consisted of a Beckman model 110A pump with a model 153 UV detector, a Rheodyne 7125 injector with a 20 μ m loop, and a Hewlett-Packard 3392A integrator. The column was a Lichrosorb RP-8 10 μ m reversed-phase column, 250 mm x 4.6 mm (inside

diameter). The mobile phase contained 10% methanol and 90% 0.025 M acetate buffer (pH=5.0) and was run at 1.0 mL/min. The column effluent was monitored at 254 nm, and quantitation was based on peak areas. Standards chromatographed under the same conditions were used for calibration.

Hydrolysis was initiated by adding 0.4 mL of a stock solution of compound **1** in acetonitrile to 25 mL of buffer prewarmed to 32 °C in a constant temperature water bath to give final concentrations of $\sim 1.8 \times 10^{-4}$ M. Aliquots were removed at appropriate intervals and chromatographed immediately. Pseudo-first-order rate constants were determined from the expression:

$$\ln(C_t) = \ln(C_0) - kt \quad (4)$$

where C_t is the concentration at some time t , C_0 is the concentration at $t=0$, k is the pseudo-first-order rate constant, and t is the time. The slopes, $-k$, of linear plots of $\ln(C_t)$ versus t were determined by linear regression. The half-lives ($t_{1/2}$) were calculated from

$$t_{1/2} = 0.693/k \quad (5)$$

Each hydrolysis reaction was run in triplicate and was followed for a minimum of three half-lives. The correlation coefficients were ≥ 0.999 .

Skin Penetration Studies

Diffusion cell experiments were performed to measure the transdermal delivery of 5FU and the 5FU prodrugs. Franz-type

diffusion cells from Crown Glass in Somerville, NJ with 4.9 cm² donor surface areas and 20 mL receptor phase volumes were used for this purpose. The full-thickness skins were obtained from female hairless mice (SKH-hr-1) from Temple University Skin and Cancer Hospital.

The mice were killed by cervical dislocation, their skins were removed immediately by blunt dissection, and dorsal sections were mounted in the diffusion cells. The dermal sides of the skins were placed in contact with receptor phase which contained 0.05 M phosphate buffer (pH=7.1, I=0.12) with 0.11% formaldehyde as a preservative. The effectiveness of formaldehyde for this purpose has recently been documented.⁸⁹ The receptor phases were stirred continuously and kept at constant temperature (32 °C) by a circulating water bath. A preapplication period of 48 hours was established to uniformly condition the skins and to remove water-soluble UV-absorbing materials. The receptor phases were changed three times during this period, and control experiments from earlier studies have shown that this procedure effectively removes those materials.⁹⁷ The epidermal sides of the skins were exposed to the air and were left untreated during this period.

After the preapplication period, 0.5 mL aliquots from suspensions of the prodrugs in IPM were applied to the epidermal sides of the skins. The IPM suspensions were stirred at 22±1 °C for 48 hours prior to application to ensure that saturation was attained. Total concentrations of

the IPM suspensions ranged from 0.3 M to 0.8 M with enough excess solid present to maintain saturation for the duration of the application period (see below). Each drug-vehicle combination was run in triplicate.

Samples were taken from the receptor phases at 4, 8, 12, 21, 24, 27, 30, 33, 36, 45, and 48 hours after donor phase application. The receptor phases were changed following removal of each sample so that "sink" conditions were maintained. Samples were analyzed for total 5FU species that had diffused by UV spectroscopy ($\epsilon=7.13 \times 10^3$ at 266 nm) after allowing at least 72 hours for complete prodrug hydrolysis. Cumulative amounts of total 5FU species that diffused (μmol) were plotted against time (h), and the slopes of the linear, "steady-state" regions were calculated using linear regression. The slopes, when divided by 4.9 (the area of the donor surface in cm^2), gave the "steady-state" fluxes ($\mu\text{mol}/\text{cm}^2/\text{h}$). In a separate experiment, HPLC was used to determine intact prodrug content in the receptor phases at each sampling time. Mobile phase containing 18-50% methanol in 0.025 M acetate buffer (pH=5.0) was used with the system described earlier. Aliquots were removed and chromatographed immediately after the samples were taken. Prodrug fluxes were calculated in the same manner as fluxes for total 5FU.

Donor phases were changed every twelve hours and were set aside for ^1H NMR analysis. Stability of the prodrugs in IPM was determined from the chemical shift of $\text{C}^6\text{-H}$. In dimethylsulfoxide- d_6 , the $\text{C}^6\text{-H}$ signal for 5FU appears at

$\delta=7.73$ ppm. For each of the 1-alkyloxycarbonyl derivatives, the same signal in dimethylsulfoxide- d_6 appears at $\delta>8.10$ ppm. Since this area of the spectrum is free from interference by IPM absorbances, the two signals can be identified and quantified if necessary.

Following removal of the donor phases after the 48-hour application period, the epidermal sides of the skins were washed three times with 5 mL portions of methanol to remove all remnants of prodrug and vehicle from the skin surfaces. This was accomplished quickly (<3 min) to minimize contact time between the skins and methanol. The receptor phases were changed again, and the dermal sides were kept in contact with the fresh buffer for 23 hours while the epidermal sides were again left exposed to the air. After this "leaching" period, another sample was taken from each cell to measure the skin accumulation of total 5FU species.

Second applications to the epidermal sides of the skins were made after the "leaching" period with a standard drug-vehicle suspension. Theophylline in propylene glycol (0.4 M) was applied to assess the damage to the skins from application of the initial drug-vehicle combinations. Samples were taken at 1, 2, 3, 5, 7, 9, and 11 or 12 hours after application. The samples were analyzed for theophylline by UV spectroscopy ($\epsilon=1.02 \times 10^4$ at 271 nm) and second application fluxes were determined as described above.

Results and Discussion

Synthesis and Structure Determination

The known 1-alkyloxycarbonyl-5FU derivatives have melting points^{25,30,93} and spectral properties (UV³⁰ and ¹H NMR²⁵) in agreement with those reported in the literature. The structures of the novel compounds were assigned by comparison of their spectral properties with those of the known homologs. Elemental microanalyses were obtained for the novel compounds and were within acceptable limits ($\pm 0.4\%$).

Acylation on the N¹- or N³-position can be distinguished by UV and ¹H NMR analysis. Anions of N³-substituted derivatives undergo a substantial shift of their UV_{max} to longer wavelength while anions of N¹-substituted compounds do not.^{30,98} This is reportedly due to the extended conjugation possible for the N¹-anion.²⁰ Differences in ¹H NMR spectra are also well defined. In chloroform-d, the C⁶-H signal for N¹-substituted derivatives is a sharp doublet indicating coupling with C⁵-F. The same signal in chloroform-d for N³-substituted derivatives appears as a broad singlet or triplet-like doublet of doublets from additional coupling of C⁶-H with N¹-H.²⁰ When the substituents contain a carbonyl group attached to the N¹-position, as they do in the 1-alkyloxycarbonyl series, an anisotropic effect is observed

in which the C⁶-H signal is shifted downfield relative to 5FU or the N³-substituted derivatives.²⁵ For example, the C⁶-H chemical shift for 1-ethyloxycarbonyl-5FU ($\delta=8.00$) is 0.77 ppm downfield when compared with 3-ethyloxycarbonyl-5FU ($\delta=7.23$) in chloroform-d.

Solubility

Solubility determinations are generally accomplished by stirring excess solute in a solvent until saturation is attained. The excess solid is removed and the saturated solution is assayed for solute content. This approach is suitable for stable solutes and for unstable solutes in aprotic solvents, but another method is needed for measuring aqueous solubilities of chemically unstable compounds.

An alternative to the direct method for determining aqueous solubilities is the partitioning-based method. The advantage of this method is that contact time between the unstable compound and the aqueous phase can be minimized. However, several points regarding this procedure require clarification.

First, partition coefficients are concentration dependent except when compounds with low associating tendencies are present in dilute solutions ($<10^{-1}$ M).⁹⁶ Solubilities based on partition coefficients can only be reported as estimates since activity coefficients become more important at higher solute concentrations.⁹⁹

Second, the partitioning and phase separation times that were used in the partitioning-based method for estimating aqueous solubility were chosen empirically. The times had to be sufficiently long to allow equilibrium distribution of the compounds between the phases to occur and to allow subsequent separation of the phases to occur without substantial hydrolysis of the prodrugs. Longer times than those chosen could have been used for these more stable 1-alkyloxycarbonyl derivatives, but in order to validate the procedure for all four series, shorter times that were more appropriate for the less stable derivatives were used.

Finally, due to the experimental design and the lability of the prodrugs, mutual saturation of the phases prior to partitioning could not be accomplished. Since the ester (IPM) that was used as the lipid phase in these experiments is practically insoluble in water,⁹⁴ changes in the phase volumes during partitioning are probably insignificant. This potential volume change is a common source of error when more water-soluble lipid solvents are not presaturated with their corresponding aqueous phases.¹⁰⁰

The conventional, direct method was used for determining lipid solubilities. Since the 1-alkyloxycarbonyl derivatives are the most chemically stable compounds of the four series, both the direct and the partitioning-based methods were used for determining their aqueous solubilities. Thus, the two methods for determining aqueous solubility were compared using the 1-alkyloxycarbonyl derivatives as a model series.

Lipid (S_{IPM}) and aqueous (S_{AQ}) solubilities for the 1-alkyloxycarbonyl derivatives are presented in Table 2-2 along with their melting points. Lipid solubilities are greatly enhanced by making derivatives. Solubility values range from over 40 times greater than 5FU for 1-methyloxycarbonyl-5FU (**1**) to more than 3000 times greater than 5FU for 1-hexyloxycarbonyl-5FU (**5**). Increases in lipid solubility with increasing chain length are accompanied by decreases in melting point. A change in that trend is observed for 1-octyloxycarbonyl-5FU (**6**), but this is not unexpected. Since melting point and solubility depend in part on crystal lattice energies,¹⁰¹ this result indicates that the crystal structure is dominated by the 5FU nucleus for lower homologs and by the aliphatic side chain for higher homologs.

Aqueous solubilities are reported for both the direct and partitioning methods. The results show that aqueous solubility peaks for 1-ethyloxycarbonyl-5FU (**2**) and then decreases. Compounds **1** and **2** have aqueous solubilities greater than 5FU even though a hydrogen-bonding group (N^1-H) has been masked. Again, this is reflected in the lowered melting points for the derivatives when compared to 5FU.

Aqueous solubilities determined by the partitioning-based method underestimated the direct solubilities by 7% for compounds **5** and **6**, 8% for compound **1**, 21% for compound **4**, 22% for compound **3**, and 34% for compound **2**. Relative aqueous solubility among members of the series is the same with

Table 2-2. Melting points (MP), lipid solubilities (S_{IPM}), and aqueous solubilities (S_{AQ}) for 1-alkyloxycarbonyl derivatives.

Compound	MP (°C)	S_{IPM}^a (mM)	S_{AQ}^b (mM)	S_{AQ}^c (mM)	S_{AQ}^d (mM)
5FU	280-2	0.049	96	-	85
1	158-60	2.1	120	111	124
2	127-8	13	263	174	34
3	124-6	15	55	43	-
4	97-8	34	29	23	26
5	66-7	153	5.4	5.0	5.8
6	97-8	36	0.14	0.13	-

^aStandard deviations from the mean were within $\pm 3\%$ for IPM solubilities.

^bStandard deviations from the mean were within $\pm 8\%$ for aqueous solubilities determined by direct method.

^cStandard deviations from the mean were within $\pm 6\%$ for experimental values used to calculate partition coefficients and estimated aqueous solubilities ($\pm 11\%$ for compound 2).

^dLiterature values from references 30 and 93.

Table 2-3. Solubility ratios (SR), partition coefficients (PC), and hydrophobicity parameters (π) for l-alkyloxycarbonyl derivatives.

Compound	SR ^a	log (SR)	π^b	PC ^c	log (PC)	π^d	log (PC) - log (SR)
1	0.018	-1.75	-	0.019	-1.72	-	0.03
2	0.050	-1.30	0.45	0.075	-1.12	0.60	0.18
3	0.28	-0.56	0.74	0.36	-0.45	0.67	0.11
4	1.2	0.07	0.63	1.4	0.16	0.61	0.09
5	29	1.46	0.70	31	1.48	0.66	0.02
6	257	2.41	0.48	285	2.45	0.49	0.04

^aSolubility ratio calculated from S_{IPM}/S_{AQ} .

^b Δ log (SR) for compound and preceding compound.

^cExperimental partition coefficient (C_{IPM}/C_{AQ}).

^d Δ log (PC) for compound and preceding compound.

either procedure, and overall, agreement between the two methods is good.

The direct aqueous solubility values for compounds **1**, **4**, and **5** agree with the corresponding literature values included in Table 2-2. However, the value for compound **2** is much higher than the literature value. The basis for this discrepancy is not clear.

In Table 2-3, the solubility ratios (SR) and experimentally determined partition coefficients (PC) are compared for the 1-alkyloxycarbonyl series. The values for $\log(PC) - \log(SR)$ indicate that the partition coefficients are somewhat higher than the corresponding solubility ratios. Generally, the more polar derivatives ($\log[PC] < 0$) show the greatest difference with the exception of compound **1**.

Yalkowsky et al.¹⁰² studied solubility ratios and octanol-water partition coefficients for a broad range of solutes. They concluded that self-association of polar solutes in octanol increases the ability of octanol to accommodate the solute which increases the partition coefficient. Conversely, a nonpolar solute causes a decrease in the partition coefficient by self-associating in the aqueous phase.¹⁰² The present results can be explained on the same basis. The lower than expected $\log(PC) - \log(SR)$ value for compound **1** could be due to the low concentration in the IPM phase during partitioning of this derivative. Since the aqueous solubility of compound **1** is nearly 60 times its lipid solubility, the concentration in the IPM phase is reduced

well below its already low concentration at saturation. Therefore, the solute-solute interactions that lead to higher partition coefficients would also be reduced.

The hydrophobicity parameters¹⁰⁰ (π) in Table 2-3 were calculated from the relationship:¹⁰³

$$\log(PC)_n = \log(PC)_0 + \pi n \quad (6)$$

where n is the number of methylene units in a homologous series using both $\log(PC)$ and $\log(SR)$ values. Both calculations yield an average π value equal to 0.60. Values for π from the literature include 0.54 for silicone oil-water and 0.66 for hexane-water¹⁰¹ indicating that 0.60 is a reasonable value for the IPM-buffer partitioning system.

Hydrolysis Kinetics

Hydrolysis of 1-methyloxycarbonyl-5FU (**1**) to 5FU in 0.05 M phosphate buffer (pH=7.1, I=0.12) with and without 0.11% formaldehyde (3.6×10^{-2} M) was followed by HPLC at 32 °C. Disappearance of compound **1**, indicated by $\ln(C)$, is plotted versus time (min) in Figure 2-1. The linearity of the plots suggests that hydrolysis of **1** follows first-order kinetics in the presence and absence of formaldehyde. Pseudo-first-order rate constants (k) and half-lives ($t_{1/2}$) from the linear plots are presented in Table 2-4.

Hydrolysis of compound **1** is clearly faster in the presence of formaldehyde indicating that general base catalysis by formaldehyde hydrate may be involved (see

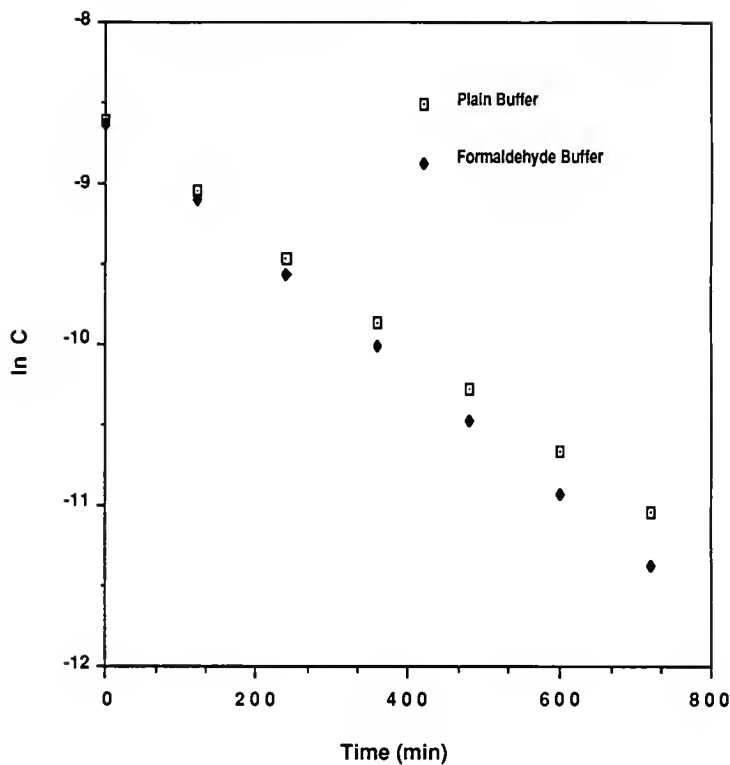


Figure 2-1. Plots of $\ln(C)$ versus time (min) for hydrolysis of 1-methyloxycarbonyl-5FU in 0.05 M phosphate buffer (pH=7.1, $I=0.12$) with and without formaldehyde at 32 °C.

Table 2-4. Pseudo-first-order rate constants (k) and half-lives ($t_{1/2}$) for hydrolysis of 1-methyloxycarbonyl-5FU in 0.05 M phosphate buffer (pH=7.1, I=0.12) with and without formaldehyde at 32 °C.

Compound	Formaldehyde (M)	k ($\pm$ SD) ^a (min ⁻¹)	$t_{1/2}$ (min)
1	0	3.33×10^{-3} (0.04×10^{-3})	208
1	3.6×10^{-2}	3.78×10^{-3} (0.05×10^{-3})	183

^aMean $\pm$ standard deviation for n=3 values.

Chapter 3). Significant buffer catalysis by phosphate has also been demonstrated for compounds in this series, and the reader is referred to the work of Buur and Bundgaard³⁰ for complete pH-rate profiles and probable hydrolysis mechanisms.

In any case, release of the parent drug from compound 1 by chemical means is too slow for dermal delivery purposes. Other members of the series would also appear to be poor candidates, since they hydrolyze chemically over two times slower than compound 1.^{30,93} In 80% human plasma, however, hydrolysis rates are much faster ($t_{1/2}$ =2-3 min)^{30,93} suggesting enzyme catalysis of the 1-alkyloxycarbonyl series. Since the skin is metabolically active, further study of these compounds in diffusion cells was warranted.

Skin Penetration

Skin penetration data from the diffusion cells are plotted as cumulative amount of total 5FU species that diffused (μmol) versus time (h). In Figure 2-2, results for 1-methyloxycarbonyl-5FU (1), 1-ethyloxycarbonyl-5FU (2), and 1-propyloxycarbonyl-5FU (3) are compared to 5FU itself. In Figure 2-3, results for 1-butyloxycarbonyl-5FU (4), 1-hexyloxycarbonyl-5FU (5), and 1-octyloxycarbonyl-5FU (6) are compared to 5FU. Error bars correspond to the standard deviation from the mean for $n=3$ values.

Fluxes (J), lag times (t_L), and skin accumulation (SA) values for each compound are reported in Table 2-5. Lag time

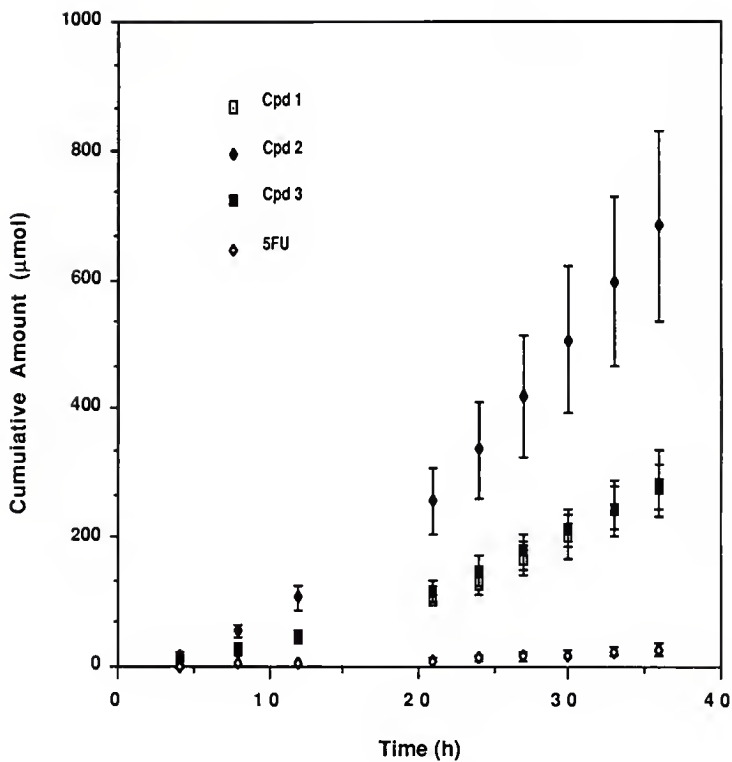


Figure 2-2. Plots of cumulative amount of total 5FU species that diffused (μmol) versus time (h) for compounds 1, 2, 3, and 5FU.

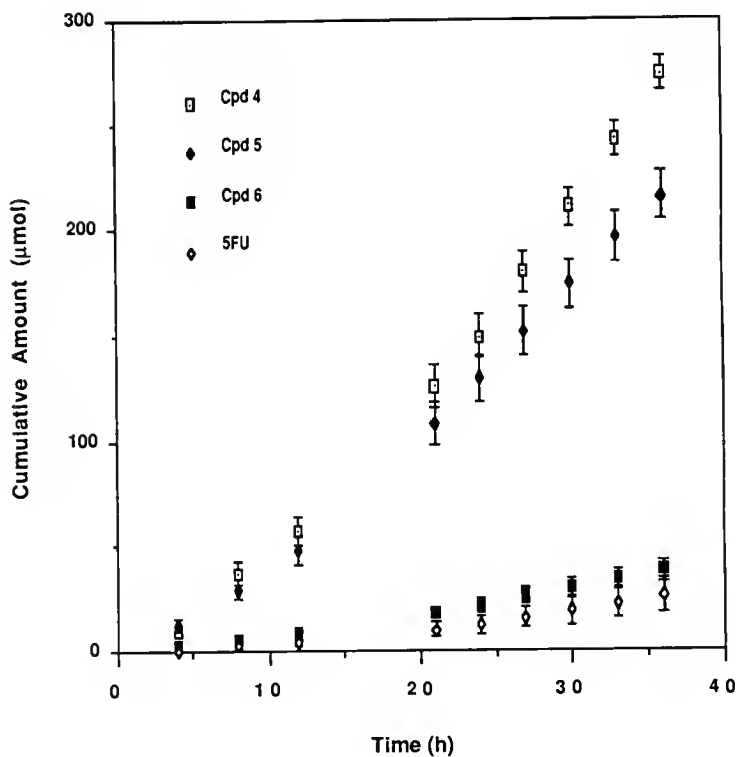


Figure 2-3. Plots of cumulative amount of total 5FU species that diffused (μmol) versus time (h) for compounds 4, 5, 6, and 5FU.

Table 2-5. Fluxes (J), lag times (t_L), and skin accumulation (SA) values for 1-alkyloxycarbonyl derivatives.

Compound	J($\pm$ SD) ^a ($\mu\text{mol}/\text{cm}^2/\text{h}$)	Prodrug S-S ^b (11 h) ^c (%)	t_L (h)	SA($\pm$ SD) ^a (μmol)
5FU	0.24(0.09)	-	13	3.7(0.9)
1	2.6(0.6)	42(16)	14	8.3(0.1)
2	5.9(1.3)	90(75)	13	18(4)
3	2.3(0.2)	78(43)	11	5.0(1.4)
4	2.2(0.1)	73(32)	10	4.2(0.5)
5	1.5(0.1)	79(14)	6.2	11(0)
6	0.29(0.02)	-	8.7	3.2(0.5)

^aMean $\pm$ standard deviation for n=3 values.

^bPercent of total 5FU as intact prodrug during "steady-state" phase in separate experiment (n=1).

^cPercent of total 5FU as intact prodrug from 11 h sample in separate experiment (n=1).

Table 2-6. Second application fluxes (J) and lag times (t_L) for 1-alkyloxycarbonyl derivatives.

Compound	$J^a (\pm SD)^b$ ($\mu\text{mol}/\text{cm}^2/\text{h}$)	t_L (h)
5FU	1.2(0.2)	1.2
1	1.9(0.1)	0.8
2	1.8(0.4)	0.9
3	1.7(0.3)	1.0
4	1.8(0.2)	1.0
5	1.8(0.1)	0.8
6	1.8(0.2)	0.9

^aFlux of 0.4 M theopylline from propylene glycol.

^bMean $\pm$ standard deviation for n=3 values.

refers to the intersection of the linear, or "steady-state," region of each graph with the time (x) axis, and it is the time required for establishing a uniform concentration gradient within the skin.⁶⁵ The percent of total 5FU present as intact prodrug in the receptor phase is also reported in Table 2-5. These values were calculated from samples taken during the "steady-state" phase and from an earlier sample (11 h) in a separate experiment using HPLC analysis (n=1).

The improvement in skin penetration of 5FU from the 1-alkyloxycarbonyl derivatives is significant except for 1-octyloxycarbonyl-5FU (6). Increases in flux are generally about one order of magnitude, and 1-ethyloxycarbonyl-5FU (2) with nearly a 25-fold improvement is easily the best derivative.

The presence of large amounts of intact prodrugs in the receptor phases is a matter of interest. The high percentages indicate that the hydrolytic enzymes of the skin are not effectively converting the prodrugs to 5FU. It is interesting to note, however, that percentages calculated from a sample removed prior to "steady-state" are much lower than the "steady-state" values. It is possible that the large amounts of diffusant present at "steady-state" are simply too much for the enzymes to handle. Another possibility is that the continuous changing of the receptor phase with each sample eventually depletes the enzymatic activity.

The trend in skin accumulation is similar to the trend in flux with the exception of 1-hexyloxycarbonyl-5FU (5). The large skin accumulation value and short lag time for compound 5 may indicate a high affinity for the lipid regions of the skin but less affinity for the hydrated regions and the receptor phase.

Second application fluxes and lag times are presented in Table 2-6. Skin penetration by theophylline from propylene glycol, the standard drug-vehicle combination, is approximately one and one-half times higher for the skins treated with the 1-alkyloxycarbonyl derivatives than for those treated with 5FU, and it is consistent throughout the series. Therefore, skin damage is greater with the prodrugs, but the difference is small when compared with the general improvement in delivery of 5FU from the prodrugs.

The stability of the prodrugs in the IPM formulations was assessed by ^1H NMR analysis of the donor phases. After a minimum of five days from the time the suspensions were prepared until their ^1H NMR spectra were recorded, including at least twelve hours during which the formulations were in contact with the skins, the 1-alkyloxycarbonyl derivatives were found to be intact with no evidence of 5FU formation.

Summary

The 1-alkyloxycarbonyl derivatives of 5FU exhibited decreased melting points and increased lipid solubilities

when compared to 5FU. Aqueous solubility reached a maximum for 1-ethyloxycarbonyl-5FU (2) and decreased from there with increasing chain length. Skin penetration and skin accumulation were also maximized for compound 2 suggesting that both lipid and aqueous solubilities are important for predicting transdermal and dermal delivery of these 5FU prodrugs. The presence of high percentages of prodrugs in the receptor phases, indicating insufficient release of the parent drugs in the hairless mouse skin model, may limit the potential of this series of prodrugs at least for dermal delivery purposes. Finally, the partitioning-based method for determining aqueous solubility appears to be a useful method particularly for determining relative solubilities in an homologous series.

CHAPTER 3 1-ACYL DERIVATIVES

Introduction

The 1-acyl derivatives of 5-fluorouracil (5FU) are chemically unstable in aqueous solutions at all pH values. In fact, their lability has been cited as a limitation to their usefulness as drugs or prodrugs.^{26,42} If properly formulated in an aprotic vehicle, however, this series of 5FU derivatives may have potential for use as prodrugs for dermal delivery.

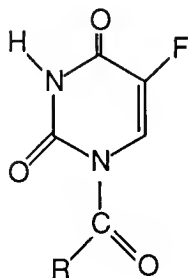
Six straight-chain 1-acyl derivatives were selected for study. The derivatives and their structures are shown in Table 3-1.

Materials and Methods

Synthesis

Melting points (mp) were determined with a Thomas-Hoover capillary melting point apparatus and are uncorrected. Elemental microanalyses were obtained for all novel compounds through Atlantic Microlab, Incorporated in Norcross, Georgia. Proton nuclear magnetic resonance (¹H NMR) spectra were obtained at 90 MHz on a Varian EM-390 spectrometer. Chemical

Table 3-1. Structures of 1-acyl derivatives.



Compound	R
1-acetyl-5FU (7)	-CH ₃
1-propionyl-5FU (8)	-CH ₂ CH ₃
1-butyryl-5FU (9)	-(CH ₂) ₂ CH ₃
1-valeryl-5FU (10)	-(CH ₂) ₃ CH ₃
1-hexanoyl-5FU (11)	-(CH ₂) ₄ CH ₃
1-octanoyl-5FU (12)	-(CH ₂) ₆ CH ₃

shifts (δ) are reported in parts per million (ppm) from the internal standard, tetramethylsilane (TMS). Coupling constants (J) are expressed in cycles per second (Hz). Infrared (IR) spectra were recorded with a Perkin-Elmer 1420 spectrophotometer and absorbances are reported in cm^{-1} . Ultraviolet (UV) spectra were obtained with a Cary 210 or Shimadzu UV-265 spectrophotometer. Maximum absorbances are reported in nm along with the molar absorptivities (ϵ) in L/mol. Single-crystal X-ray analysis was obtained for 1-acetyl-5FU through Hoffmann-La Roche in Nutley, NJ.

1-Acyl-5-fluorouracil (general procedure)

To 0.66 g (0.01 mol) of 85% potassium hydroxide dissolved in methanol (20-50 mL) was added 1.31 g of 5-fluorouracil (0.01 mol). The methanol suspension was stirred for 30 minutes, and the methanol was evaporated under reduced pressure. The potassium salt was suspended in acetonitrile (25-50 mL) which was evaporated under reduced pressure to remove residual methanol. The salt was resuspended in acetonitrile (25-50 mL), and the suspension was added dropwise over 15 to 30 minutes to a well stirred acetonitrile (25 mL) solution in an ice bath containing 1.0 to 1.2 equivalents of the appropriate acid chloride. The mixture was stirred at 0 °C for 60 minutes. The mixture was filtered, and the residue was washed with acetonitrile (25 mL). The combined acetonitrile solutions were evaporated under reduced pressure, and the solid residue was

crystallized from an appropriate solvent or solvent combination.

1-Acetyl-5-fluorouracil (7)

Crystallization from dichloromethane gave 0.98 g of **7** (57%): mp 129-30 °C (lit.²⁷ mp 126-7 °C); IR (KBr) 1670, 1695, 1725, and 1770 cm^{-1} (C=O); ^1H NMR (CDCl_3) δ 2.73 (s, 3H, CH_3) and 8.23 (d, $J=7$ Hz, 1H, $\text{C}^6\text{-H}$); UV_{max} (CH_3CN) 261 nm ($\epsilon=1.125 \times 10^4$).

1-Propionyl-5-fluorouracil (8)

Crystallization from dichloromethane/hexane gave 1.32 g of **8** (71%): mp 130-1 °C (lit.²⁷ mp 124-5 °C); IR (KBr) 1695, 1710, and 1740 cm^{-1} (C=O); ^1H NMR (CDCl_3) δ 1.25 (t, $J=7$ Hz, 3H, CH_3), 3.14 (q, $J=7$ Hz, 2H, COCH_2), and 8.27 (d, $J=7$ Hz, 1H, $\text{C}^6\text{-H}$); UV_{max} (CH_3CN) 261 nm ($\epsilon=1.141 \times 10^4$).

Anal. Calc. for $\text{C}_7\text{H}_7\text{FN}_2\text{O}_3$: C, 45.17; H, 3.79; N, 15.05.
Found: C, 45.26; H, 3.83; N, 14.97.

1-Butyryl-5-fluorouracil (9)

Crystallization from dichloromethane/hexane gave 0.86 g of **9** (43%): mp 145-6 °C; IR (KBr) 1690, 1710, and 1740 cm^{-1} (C=O); ^1H NMR (CDCl_3) δ 1.01 (t, $J=7$ Hz, 3H, CH_3), 1.77 (m, 2H, COCH_2CH_2), 3.09 (t, $J=7$ Hz, 2H, COCH_2), and 8.25 (d, $J=6$ Hz, 1H, $\text{C}^6\text{-H}$); UV_{max} (CH_3CN) 261 nm ($\epsilon=1.168 \times 10^4$).

Anal. Calc. for $\text{C}_8\text{H}_9\text{FN}_2\text{O}_3$: C, 48.00; H, 4.53; N, 14.00.
Found: C, 48.12; H, 4.58; N, 13.91.

1-Valeryl-5-fluorouracil (10)

Crystallization from dichloromethane/hexane gave 1.35 g of **10** (63%): mp 120-1 °C; IR (KBr) 1695, 1715, and 1740 cm^{-1} (C=O); ^1H NMR (CDCl_3) δ 0.95 (t, $J=7$ Hz, 3H, CH_3), 1.3-1.8 (m, 4H, $\text{COCH}_2\text{CH}_2\text{CH}_2$), 3.11 (t, $J=7$ Hz, 2H, COCH_2), and 8.24 (d, $J=7$ Hz, 1H, $\text{C}^6\text{-H}$); UV_{max} (CH_3CN) 261 nm ($\epsilon=1.175 \times 10^4$).

Anal. Calc. for $\text{C}_9\text{H}_{11}\text{FN}_2\text{O}_3$: C, 50.47; H, 5.18; N, 13.08.
Found: C, 50.52; H, 5.23; N, 13.03.

1-Hexanoyl-5-fluorouracil (11)

Crystallization from dichloromethane/hexane gave 1.71 g of **11** (75%): mp 101-2 °C; IR (KBr) 1690, 1715, and 1745 cm^{-1} (C=O); ^1H NMR (CDCl_3) δ 0.92 (distd t, 3H, CH_3), 1.2-1.9 (m, 6H, $\text{COCH}_2\text{CH}_2\text{CH}_2\text{CH}_2$), 3.09 (t, $J=7$ Hz, 2H, COCH_2), and 8.24 (d, $J=7$ Hz, 1H, $\text{C}^6\text{-H}$); UV_{max} (CH_3CN) 261 nm ($\epsilon=1.158 \times 10^4$).

Anal. Calc. for $\text{C}_{10}\text{H}_{13}\text{FN}_2\text{O}_3$: C, 52.63; H, 5.74; N, 12.27.
Found: C, 52.69; H, 5.75; N, 12.27.

1-Octanoyl-5-fluorouracil (12)

Crystallization from dichloromethane/hexane gave 1.28 g of **12** (50%): mp 83-4 °C; IR (KBr) 1685, 1710, and 1745 cm^{-1} (C=O); ^1H NMR (CDCl_3) δ 0.90 (distd t, 3H, CH_3), 1.2-1.8 (m, 10H, $\text{COCH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2$), 3.10 (t, $J=7$ Hz, 2H, COCH_2), and 8.23 (d, $J=7$ Hz, 1H, $\text{C}^6\text{-H}$); UV_{max} (CH_3CN) 261 nm ($\epsilon=1.155 \times 10^4$).

Anal. Calc. for $\text{C}_{12}\text{H}_{17}\text{FN}_2\text{O}_3$: C, 56.24; H, 6.69; N, 10.93.
Found: C, 56.22; H, 6.73; N, 10.96.

Lipid solubility

Lipid solubilities were determined using isopropyl myristate (IPM), a commercial vehicle used in cosmetics and topical medicinals,⁹⁴ as the lipid solvent. The use of IPM as a model lipophilic vehicle in skin penetration studies is well established.^{77,95}

Three suspensions of each derivative were stirred at 22 ± 1 °C for 48 hours. The suspensions were filtered through 0.45 μm nylon filters, and the saturated solutions were diluted in acetonitrile and analyzed by UV spectroscopy. Solubilities were calculated using Beer's Law:

$$A = \epsilon \cdot C \cdot d \quad (1)$$

where A is the absorbance, ϵ is the molar absorptivity, C is the concentration in mol/L, and d is the path length of the cuvette in cm. Molar absorptivities were predetermined in triplicate in acetonitrile at 261 nm.

Aqueous Solubility

Because of the chemical instability of the 1-acyl derivatives, direct measurement of aqueous solubilities for these prodrugs was not attempted. A comparison of the direct and partitioning-based methods for determining aqueous solubility was presented in Chapter 2 for the 1-alkyloxy-carbonyl derivatives.

Partition Coefficients

The partitioning-based method for determining aqueous solubility utilized the saturated IPM solutions from the lipid solubility study. For most compounds, equal volumes (1 mL) of saturated IPM solution and 0.05 M acetate buffer (pH=4.0) were used. The use of equal or near-equal phase volumes is known to facilitate rapid equilibrium.⁹⁶ The two phases were mixed thoroughly for ten seconds and allowed to separate for 60 seconds. A preliminary study with 1-acetyl-5FU (7) showed that there was virtually no difference in partition coefficient (PC) values when partitioning was carried out for 10, 20, or 30 seconds ($PC=0.183\pm0.006$, a standard deviation of only 3%). The IPM layers were diluted in acetonitrile and analyzed by UV spectroscopy. The IPM-buffer partition coefficients were calculated as follows:

$$PC = A_{\text{after}} / (A_{\text{before}} - A_{\text{after}}) \cdot V_{\text{AQ}} / V_{\text{IPM}} \quad (2)$$

where A_{after} is the absorbance from the IPM layer after partitioning, A_{before} is the absorbance from the IPM layer before partitioning, V_{AQ} is the volume of the aqueous phase, and V_{IPM} is the volume of the IPM phase. Estimated aqueous solubilities (S_{AQ}) were calculated from the IPM solubility (S_{IPM}) and the partition coefficient:

$$S_{\text{AQ}} = S_{\text{IPM}} / PC \quad (3)$$

Partitioning was carried out in triplicate for a fixed volume ratio for each derivative. For those compounds with large

differences in solubility in one phase relative to the other, volume ratios (IPM:buffer) other than 1:1 were necessary, but the ratio never exceeded 10:1 or 1:10.

Hydrolysis Kinetics

Hydrolysis rates have previously been reported for one member of this homologous series.²⁶ In the present study, hydrolysis rates were determined at 32 °C for all six 1-acyl derivatives in 0.05 M phosphate buffer (pH=7.1, I=0.12) and for 1-acetyl-5FU (7) in the same buffer with 0.11% formaldehyde (3.6×10^{-2} M). The rate in the presence of formaldehyde was determined for comparison with the rate in plain buffer since formaldehyde was used as a preservative in the diffusion cell experiments described in the following section. Two other concentrations of formaldehyde (1.8×10^{-1} M and 3.6×10^{-1} M) were studied to assess the catalytic role of formaldehyde in the hydrolysis of compound 7.

The hydrolyses were followed by UV spectroscopy at 266 nm where the absorbance decrease accompanying hydrolysis of the 1-acyl derivatives was maximized. Hydrolysis was initiated by adding 60 to 75 μ L of stock solutions of the derivatives in acetonitrile to 3 mL of buffer prewarmed to 32 °C in a thermostated quartz cuvette to give final concentrations of $1\text{--}2 \times 10^{-4}$ M. Absorbances were recorded at appropriate intervals and pseudo-first-order rate constants

were determined from the expression:

$$\ln(A_t - A_\infty) = \ln(A_0 - A_\infty) - kt \quad (4)$$

where A_t is the absorbance at some time t , A_∞ is the absorbance at $t = \infty$, A_0 is the absorbance at $t = 0$, k is the pseudo-first-order rate constant, and t is the time. The hydrolyses were sufficiently fast to allow experimental determination of A_∞ . The slopes, $-k$, of linear plots of $\ln(A_t - A_\infty)$ versus time were determined by linear regression. The half-lives ($t_{1/2}$) were calculated from

$$t_{1/2} = 0.693/k \quad (5)$$

Each hydrolysis reaction was run in triplicate and was followed for a minimum of three half-lives. The correlation coefficients were ≥ 0.999 .

Skin Penetration Studies

Diffusion cell experiments were performed to measure the transdermal delivery of 5FU and the 5FU prodrugs. Franz-type diffusion cells from Crown Glass in Somerville, NJ with 4.9 cm² donor surface area and 20 mL receptor phase volume were used for this purpose. The full-thickness skins were obtained from female hairless mice (SKH-hr-1) from Temple University Skin and Cancer Hospital.

The mice were killed by cervical dislocation, their skins were removed immediately by blunt dissection, and dorsal sections were mounted in the diffusion cells. The dermal sides of the skins were placed in contact with

receptor phase which contained 0.05 M phosphate buffer (pH=7.1, I=0.12) with 0.11% formaldehyde as a preservative. The effectiveness of formaldehyde for this purpose has recently been documented.⁸⁹ The receptor phases were stirred continuously and kept at constant temperature (32 °C) by a circulating water bath. A preapplication period of 48 hours was established to uniformly condition the skins and to remove water-soluble UV-absorbing materials. The receptor phases were changed three times during this period, and control experiments from earlier studies have shown that this procedure effectively removes those materials.⁹⁷ The epidermal sides of the skins were exposed to the air and were left untreated during this period.

After the preapplication period, 0.5 mL aliquots from suspensions of the prodrugs in IPM were applied to the epidermal sides of the skins. The IPM suspensions were stirred at 22 ± 1 °C for 48 hours prior to application to ensure that saturation was attained. Total concentrations of the IPM suspensions ranged from 0.6 M to 1.0 M with enough excess solid present to maintain saturation for the duration of the application period (see below). Each drug-vehicle combination was run in triplicate.

Samples were taken from the receptor phases at 4, 8, 12, 21, 24, 27, 30, 33, 36, 45, and 48 hours after donor phase application. The receptor phases were changed following removal of each sample so that "sink" conditions were maintained. Samples were analyzed for total 5FU species that

had diffused by UV spectroscopy ($\epsilon=7.13 \times 10^3$ at 266 nm) after allowing at least 24 hours for complete prodrug hydrolysis. Cumulative amounts of total 5FU species that diffused (μmol) were plotted against time (h), and the slopes of the linear, "steady-state" regions were calculated using linear regression. The slopes, when divided by 4.9 (the area of the donor surface in cm^2), gave the "steady-state" fluxes ($\mu\text{mol}/\text{cm}^2/\text{h}$). Because of the rapid chemical hydrolysis of the 1-acyl derivatives, no attempt was made to analyze the receptor phases for prodrug content.

Donor phases were changed every twelve hours and were set aside for ^1H NMR analysis. Stability of the prodrugs in IPM was determined from the chemical shift of $\text{C}^6\text{-H}$. In dimethylsulfoxide- d_6 , the $\text{C}^6\text{-H}$ signal for 5FU appears at $\delta=7.73$ ppm. For each of the 1-acyl derivatives, the same signal in dimethylsulfoxide- d_6 appears at $\delta>8.20$ ppm. Since this area of the spectrum is free from interference by IPM absorbances, the two signals can be identified and quantified if necessary.

Following removal of the donor phases after the 48-hour application period, the epidermal sides of the skins were washed three times with 5 mL portions of methanol to remove all remnants of prodrug and vehicle from the skin surfaces. This was accomplished quickly (<3 min) to minimize contact time between the skins and methanol. The receptor phases were changed again, and the dermal sides were kept in contact with the fresh buffer for 23 hours while the epidermal sides

were again left exposed to the air. After this "leaching" period, another sample was taken from each cell to measure the skin accumulation of total 5FU species.

Second applications to the epidermal sides of the skins were made after the "leaching" period with a standard drug-vehicle suspension. Theophylline in propylene glycol (0.4 M) was applied to assess the damage to the skins from application of the initial drug-vehicle combinations. Samples were taken at 1, 2, 3, 5, 7, 9, and 11 or 12 hours after application. The samples were analyzed for theophylline by UV spectroscopy ($\epsilon=1.02 \times 10^4$ at 271 nm) and second application fluxes were determined as described above.

Results and Discussion

Synthesis and Structure Determination

The known 1-acyl-5FU derivatives have melting points²⁷ and ^1H NMR spectra²⁵ in agreement with those reported in the literature. The structures of novel compounds were assigned by comparison of their ^1H NMR spectra with those of the known homologs. Elemental microanalyses were obtained for the novel compounds and were within acceptable limits ($\pm 0.4\%$).

The differences in spectral properties between N^1 - and N^3 -acylated derivatives were discussed in detail in Chapter 2. Ultraviolet (UV) spectra were not obtained for the 1-acyl derivatives under basic conditions because of

their instability. However, differences in ^1H NMR spectra are very clear. In chloroform-d, the $\text{C}^6\text{-H}$ signal for 1-acetyl-5FU is a sharp doublet at $\delta=8.23$, while the same signal for 3-acetyl-5FU is a broad singlet at $\delta=7.23$, a difference of 1.00 ppm.

Results from the single-crystal X-ray analysis show that the N^1 -assignment for 1-acetyl-5FU is correct. The unit cell was found to contain two independent molecules (Figure 3-1 and Figure 3-2). Both conformations show that the carbonyl group from the 1-acyl group is positioned cis to the $\text{C}^6\text{-H}$. The circulating π electrons of carbonyl bonds are known to deshield ortho protons in structurally similar compounds such as acetophenone.¹⁰⁵ Thus, the X-ray results support the explanation that an anisotropic effect is responsible for the downfield shift of $\text{C}^6\text{-H}$ in ^1H NMR spectra of N^1 -acylated derivatives.

Solubility

Lipid (S_{IPM}) and aqueous (S_{AQ}) solubilities for the 1-acyl derivatives are presented in Table 3-2 along with their melting points. In general, melting points decrease and lipid solubilities increase with increasing chain length. An exception to both of these trends is seen for 1-butyryl-5FU (9). The basis for this variance from the observed trends is not clear.

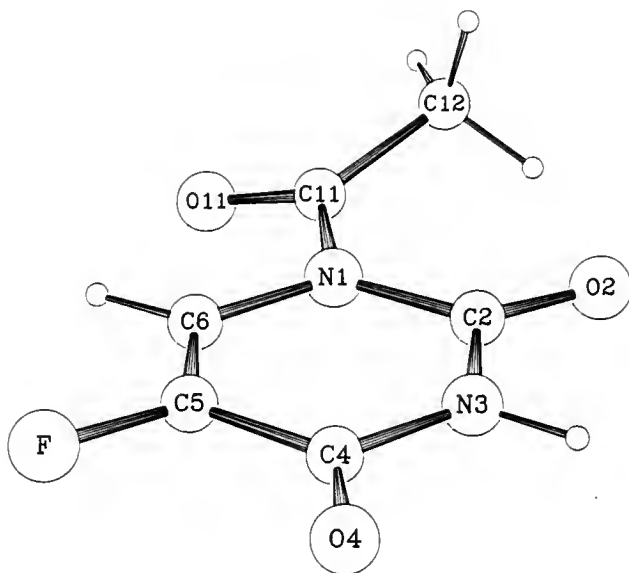


Figure 3-1. X-ray structure of 1-acetyl-5FU (unprimed).
Source: Hoffman-La Roche, Nutley, NJ.

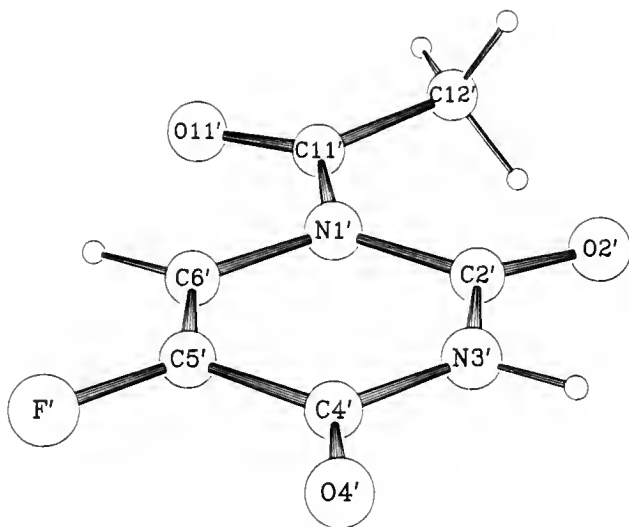


Figure 3-2. X-ray structure of 1-acetyl-5FU (primed).
Source: Hoffmann-La Roche, Nutley, NJ.

Table 3-2. Melting points (MP), lipid solubilities (S_{IPM}), and aqueous solubilities (S_{AQ}) for 1-acyl derivatives.

Compound	MP (°C)	S_{IPM}^a (mM)	S_{AQ}^b (mM)	S_{AQ}^c (mM)
5FU	280-2	0.049	96	-
7	129-30	22	-	119
8	130-1	36	-	48
9	145-6	17	-	6.5
10	120-1	39	-	3.5
11	101-2	112	-	3.0
12	83-4	111	-	0.15

^aStandard deviations from the mean were within $\pm 5\%$ for IPM solubilities.

^bSolubility determined by direct method.

^cStandard deviations from the mean were within $\pm 5\%$ for experimental values used to calculate partition coefficients and estimated aqueous solubilities.

Table 3-3. Partition coefficients (PC) and hydrophobicity parameters (π) for 1-acyl derivatives.

Compound	PC ^a	log(PC)	π ^b
7	0.19	-0.73	-
8	0.76	-0.12	0.61
9	2.7	0.43	0.55
10	11	1.05	0.62
11	38	1.58	0.53
12	759	2.88	0.65

^aExperimental partition coefficient (C_{IPM}/C_{AQ}).

^b $\Delta\log(PC)$ for compound and preceding compound.

Aqueous solubilities were determined by the partitioning-based method for which a theoretical discussion was presented in Chapter 2. For the 1-acyl series, aqueous solubility decreases with increasing chain length, and only 1-acetyl-5FU (7) is more water soluble than 5FU.

In Table 3-3, partition coefficients (PC) and hydrophobicity parameters (π) are listed for the 1-acyl derivatives. The average π value is 0.60, the same value that was calculated for the 1-alkyloxycarbonyl series. Individual values for the 1-acyl derivatives are all within 0.07 of the average. These results further support use of the partitioning-based method for determining relative aqueous solubility in a homologous series.

Hydrolysis Kinetics

Hydrolysis of the 1-acyl derivatives was studied in 0.05 M phosphate buffer (pH=7.1, I=0.12) at 32 °C. Pseudo-first-order rate constants (k) and half-lives ($t_{1/2}$) are presented in Table 3-4. Half-lives are relatively consistent throughout the series ranging from 3.1 minutes for 1-propionyl-5FU (8) to 4.8 minutes for 1-acetyl-5FU (7).

Hydrolysis of compound 7 was also studied in the same buffer with increasing concentrations of formaldehyde added to assess the catalytic role of formaldehyde hydrate. These results are shown in Table 3.5. It is clear that the hydrolysis rate for compound 7 is faster in the presence of

Table 3-4. Pseudo-first-order rate constants (k) and half-lives ($t_{1/2}$) for hydrolysis of 1-acyl derivatives in 0.05 M phosphate buffer (pH=7.1, $I=0.12$) at 32 °C.

Compound	$k (\pm SD)^a$ (min ⁻¹)	$t_{1/2}$ (min)
7	0.143 (0.002)	4.8
8	0.222 (0.004)	3.1
9	0.163 (0.003)	4.3
10	0.169 (0.006)	4.1
11	0.173 (0.003)	4.0
12	0.183 (0.003)	3.8

^aMean $\pm$ standard deviation for $n=3$ values.

Table 3-5. Pseudo-first-order rate constants (k) and half-lives ($t_{1/2}$) for hydrolysis of 1-acetyl-5FU in 0.05 M phosphate buffer (pH=7.1, I=0.12) with and without formaldehyde at 32 °C.

Compound	Formaldehyde (M)	k (±SD) (min ⁻¹)	t _{1/2} (min)
7	0	0.143(0.002) ^a	4.8
7	3.6×10 ⁻²	0.161(0.008) ^b	4.3
7	1.8×10 ⁻¹	0.219(0.004) ^a	3.2
7	3.6×10 ⁻¹	0.279(0.006) ^a	2.5

^aMean ± standard deviation for n=3 values.

^bMean ± standard deviation for n=5 values.

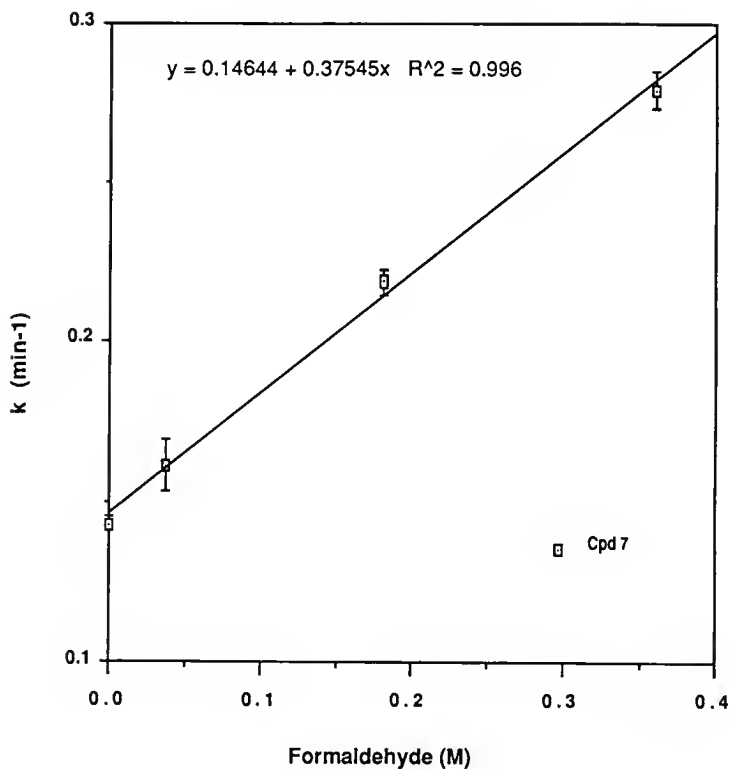


Figure 3-3. Plot of pseudo-first-order rate constant (k) versus formaldehyde concentration (M) for hydrolysis of 1-acetyl-5FU in 0.05 M phosphate buffer (pH=7.1, I=0.12) at 32 °C.

formaldehyde and the rate is concentration dependent. In Figure 3-3, the pseudo-first-order rate constants are plotted against formaldehyde concentration. The slope of the linear plot gives the catalytic rate constant ($k_{cat}=0.375$) for formaldehyde catalysis of compound **7**. Hydrolysis rates for the 1-acyl derivatives have previously been shown to be independent of pH at acidic pH values.²⁶ Therefore, formaldehyde catalysis is likely due to general base rather than general acid catalysis.

Skin Penetration

Skin penetration data from the diffusion cells are plotted as cumulative amount of total 5FU species that diffused (μmol) versus time (h). In Figure 3-4, results for 1-acetyl-5FU (**7**), 1-propionyl-5FU (**8**), and 1-butyryl-5FU (**9**) are compared to 5FU itself. In Figure 3-5, results for 1-valeryl-5FU (**10**), 1-hexanoyl-5FU (**11**), and 1-octanoyl-5FU (**12**) are compared to 5FU. Error bars correspond to the standard deviation from the mean for $n=3$ values.

Fluxes (J), lag times (t_L), and skin accumulation (SA) values for each compound are reported in Table 3-6. Lag time refers to the intersection of the linear, or "steady-state," region of each graph with the time (x) axis, and it is the time required for establishing a uniform concentration gradient within the skin.⁶⁵ The percentages of total 5FU

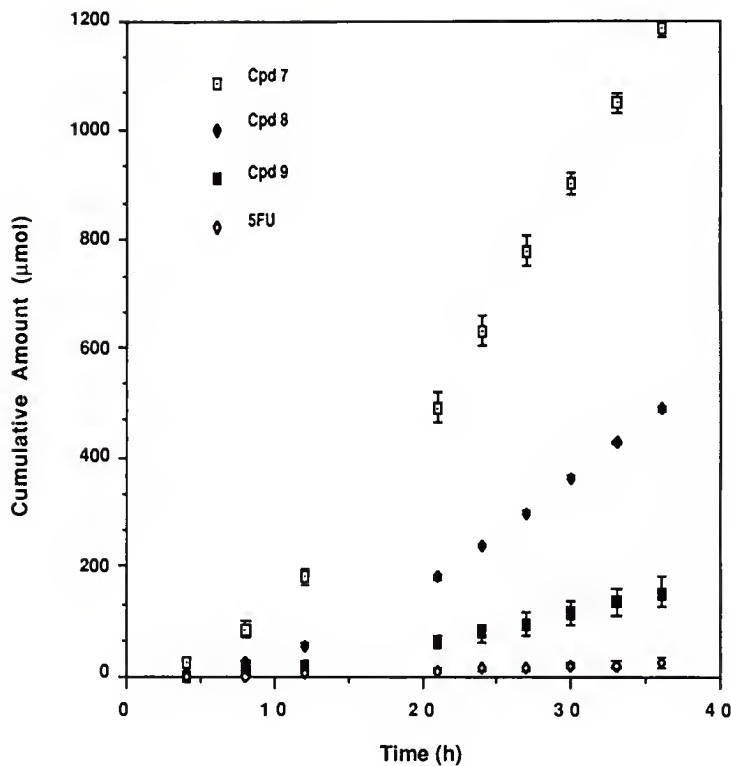


Figure 3-4. Plots of cumulative amount of total 5FU species that diffused (μmol) versus time (h) for compounds 7, 8, 9, and 5FU.

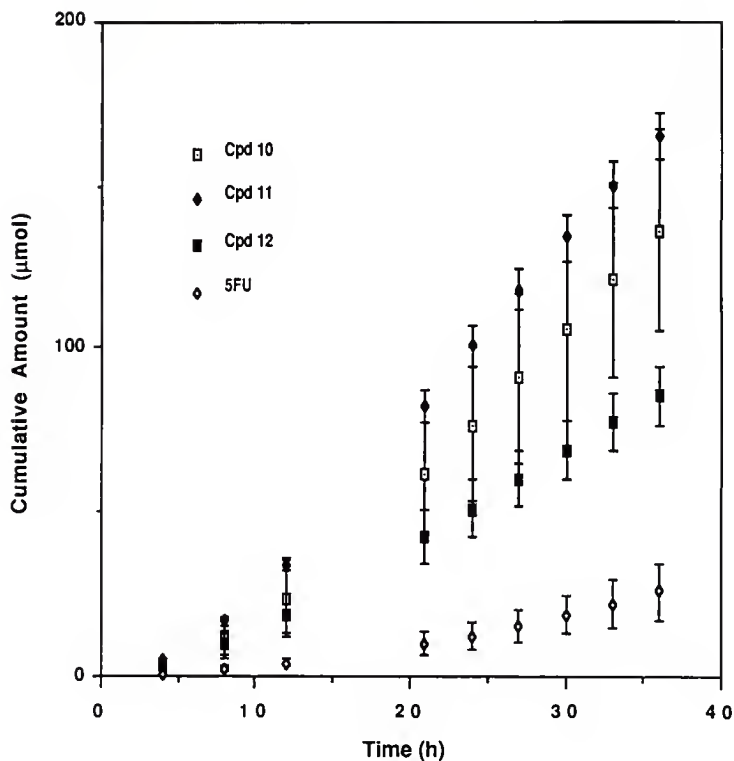


Figure 3-5. Plots of cumulative amount of total 5FU species that diffused (μmol) versus time (h) for compounds **10**, **11**, **12**, and 5FU.

Table 3-6. Fluxes (J), lag times (t_L), and skin accumulation (SA) values for 1-acyl derivatives.

Compound	J($\pm$ SD) ^a ($\mu\text{mol}/\text{cm}^2/\text{h}$)	t_L (h)	SA($\pm$ SD) ^a (μmol)
5FU	0.24(0.09)	13	3.7(0.9)
7	9.3(0.3)	10	68(10)
8	4.3(0.1)	13	69(10)
9	1.3(0.2)	12	8.2(2.7)
10	1.0(0.1)	9.0	16(4)
11	1.1(0.0)	5.6	11(3)
12	0.60(0.01)	6.5	12(3)

^aMean $\pm$ standard deviation for n=3 values.

Table 3-7. Second application fluxes (J) and lag times (t_L) for 1-acyl derivatives.

Compound	$J^a (\pm SD)^b$ ($\mu\text{mol}/\text{cm}^2/\text{h}$)	$t_L(\text{h})$
5FU	1.2 (0.2)	1.2
7	1.6 (0.0)	0.6
8	1.2 (0.2)	0.1
9	1.0 (0.0)	0.6
10	0.80 (0.03)	0.8
11	0.47 (0.02)	0.2
12	0.72 (0.11)	0.4

^aFlux of 0.4 M theopylline from propylene glycol.

^bMean $\pm$ standard deviation for n=3 values.

present as prodrug are presumed to be zero since the half-lives of these prodrugs under diffusion cell conditions are only three to five minutes.

The improvement in skin penetration of 5FU from the 1-acyl derivatives is substantial. The best compound, 1-acetyl-5FU (7), shows an increase in flux of nearly 40 times when compared to 5FU. As chain length increases, the fluxes decrease, but even 1-octanoyl-5FU (12) improves transdermal delivery of 5FU by two and one-half times.

Skin accumulation values are also much higher for the 1-acyl derivatives than for 5FU. They show a decrease with increasing chain length, but the value for 1-butyryl-5FU (9) is lower than expected. As noted earlier, the lipid solubility of compound 9 was also less than expected when compared to the compounds around it in the 1-acyl series. Therefore, the low skin accumulation value for compound 9 may be due to its low affinity for the lipid regions of the skin. Overall, skin accumulation is higher for this series than for the 1-alkyloxycarbonyl series while skin penetration data for the two series are similar. Rapid hydrolysis of the more lipid-soluble 1-acyl derivatives to highly polar 5FU as they partition into the skin may indicate that only 5FU is diffusing through the remaining lipid regions of the skin. This may effectively "lock in" large amounts of 5FU leading to high skin accumulation values.

Second application fluxes and lag times are reported in Table 3-7. Skin penetration by theophylline from propylene

glycol, the standard drug-vehicle combination, after treatment with the 1-acyl derivatives is similar to that following treatment with 5FU. In fact, the longer-chain derivatives in this series actually appear to have a protective effect on the skin since they cause less damage than 5FU itself.

The stability of the prodrugs in the IPM formulations was assessed by ^1H NMR analysis of the donor phases. After a minimum of five days from the time the suspensions were prepared until their ^1H NMR spectra were recorded, including at least twelve hours during which the formulations were in contact with the skins, the 1-acyl derivatives were found to be intact with no evidence of 5FU formation.

Summary

The 1-acyl derivatives of 5FU exhibited decreased melting points and increased lipid solubilities when compared to 5FU. Aqueous solubility reached a maximum for 1-acetyl-5FU (7) and decreased from there with increasing chain length. Skin penetration was also maximized for compound 7 while skin accumulation values were highest and essentially the same for compound 7 and 1-propionyl-5FU (8). As was also the case for the 1-alkyloxycarbonyl series, this demonstrates that both lipid and aqueous solubilities are important for predicting transdermal and dermal delivery of 5FU prodrugs. The 1-acyl derivatives may be better candidates for dermal

delivery purposes than the 1-alkyloxycarbonyl derivatives because of their rapid hydrolysis and higher skin accumulation values. The partitioning-based method for determining aqueous solubilities of chemically unstable prodrugs was used to estimate the aqueous solubilities of the 1-acyl derivatives since this could not be accomplished by other methods.

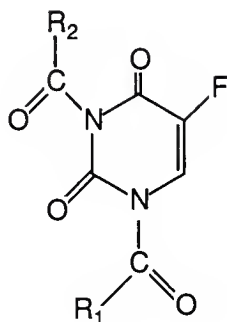
CHAPTER 4 1,3-BIS-ACYL DERIVATIVES

Introduction

The 1,3-bis-acyl derivatives of 5FU are double prodrugs. The acyl groups at the N¹-position are rapidly hydrolyzed leaving the 3-acyl derivatives. The 3-acyl derivatives will be discussed in Chapter 5. The rate of N¹-deacylation for the 1,3-bis-acyl derivatives is even faster than hydrolysis of the 1-acyl derivatives. This can best be explained by comparing pKa values for the 3-acyl derivatives and 5FU. The 3-acyl derivatives have reported pKa values of 7.1 to 7.2²⁶ while the pKa for the first ionization of 5FU is 8.0. Thus, the leaving group potential of the N³-acyl anions is greater than the 5FU anion, and the rates of N¹-deacylation are faster for the 1,3-bis-acyl derivatives.

The 1,3-bis-acyl derivatives were chosen as prodrug candidates in order to study a series in which both hydrogen-bonding groups were masked. It was anticipated that these compounds would be more lipid soluble and less water soluble than the other series and would be useful for comparison with the other series.

Table 4-1. Structures of 1,3-bis-acyl derivatives.



Compound	R_1, R_2
1,3-bis-acetyl-5FU (13)	$-\text{CH}_3$
1,3-bis-propionyl-5FU (14)	$-\text{CH}_2\text{CH}_3$
1,3-bis-butyryl-5FU (15)	$-(\text{CH}_2)_2\text{CH}_3$
1,3-bis-valeryl-5FU (16)	$-(\text{CH}_2)_3\text{CH}_3$

Four straight-chain 1,3-bis-acyl derivatives were selected for study. The derivatives and their structures are shown in Table 4-1.

Materials and Methods

Synthesis

Melting points (mp) were determined with a Thomas-Hoover capillary melting point apparatus and are uncorrected. Elemental microanalyses were obtained for all novel compounds through Atlantic Microlab, Incorporated in Norcross, Georgia. Proton nuclear magnetic resonance (^1H NMR) spectra were obtained at 90 MHz on a Varian EM-390 spectrometer. Chemical shifts (δ) are reported in parts per million (ppm) from the internal standard, tetramethylsilane (TMS). Coupling constants (J) are expressed in cycles per second (Hz). Infrared (IR) spectra were recorded with a Perkin-Elmer 1420 spectrophotometer and absorbances are reported in cm^{-1} . Ultraviolet (UV) spectra were obtained with a Cary 210 or Shimadzu UV-265 spectrophotometer. Maximum absorbances are reported in nm along with the molar absorptivities (ϵ) in L/mol. Single-crystal X-ray analysis was obtained for 1,3-bis-acetyl-5FU through Hoffmann-La Roche in Nutley, NJ.

1,3-bis-Acyl-5-fluorouracil (general procedure)

To 1.31 g (0.01 mol) of 5FU suspended in acetonitrile (20 mL) was added 1.01 g of triethylamine (0.01 mol) in

acetonitrile (5 mL). The mixture was stirred continuously at 0 °C while 0.011 mol of the appropriate acid chloride in acetonitrile (5 mL) was added dropwise over 5-10 minutes. The above sequence was repeated until 0.03 mol of triethylamine and 0.033 mol of acid chloride were added, then the mixture was stirred for an additional 30 minutes at 0 °C. Alternate addition of base and acylating agent was found to increase yield and decrease formation of colored side products that were difficult to remove. The mixture was filtered, and the residue was washed with acetonitrile (25 mL). The combined acetonitrile solutions were evaporated under reduced pressure, and the solid residue was crystallized from an appropriate solvent or solvent combination.

1,3-bis-Acetyl-5-fluorouracil (13)

Crystallization from ether gave 1.69 g of **13** (79%): mp 112-3 °C (lit.²⁵ mp 111-3 °C); IR (KBr) 1680, 1695, 1740, 1750, and 1795 cm^{-1} (C=O); ^1H NMR (CDCl_3) δ 2.58 (s, 3H, 3- CH_3), 2.72 (s, 3H, 1- CH_3), and 8.23 (d, $J=7$ Hz, 1H, C⁶-H); UV_{max} (CH_3CN) 262 nm ($\epsilon=9.75 \times 10^3$).

1,3-bis-Propionyl-5-fluorouracil (14)

Crystallization from ether gave 1.82 g of **14** (75%): mp 100-1 °C; IR (KBr) 1690, 1725, and 1795 cm^{-1} (C=O); ^1H NMR (CDCl_3) δ 1.25 (t, $J=7$ Hz, 3H, 3- CH_3), 1.28 (t, $J=7$ Hz, 3H, 1- CH_3), 2.85 (q, $J=7$ Hz, 2H, 3-COCH₂), 3.11 (q, $J=7$ Hz, 2H,

1-COCH₂), and 8.25 (d, J=7 Hz, 1H, C⁶-H); UV_{max} (CH₃CN) 262 nm ($\epsilon=9.65 \times 10^3$).

Anal. Calc. for C₁₀H₁₁FN₂O₄: C, 49.59; H, 4.58; N, 11.57.
Found: C, 49.44; H, 4.59; N, 11.53.

1,3-bis-Butyryl-5-fluorouracil (15)

Extraction of the residue with hot low-boiling petroleum ether gave a cloudy solution which cleared upon cooling to 0 °C and produced a yellow resinous sediment. The mixture was allowed to warm to room temperature, and the clear solution was decanted from the sediment. The solution was cooled again to 0 °C and crystallization gave 1.70 g of **15** (63%): mp 48-9 °C (lit.²⁶ mp 47.5-48.5 °C); IR (KBr) 1685, 1710, 1735, and 1795 cm⁻¹ (C=O); ¹H NMR (CDCl₃) δ 1.00 (t, J=7 Hz, 3H, 3-CH₃), 1.03 (t, J=7 Hz, 3H, 1-CH₃), 1.6-1.9 (m, 4H, 3-COCH₂CH₂ and 1-COCH₂CH₂), 2.80 (t, J=7 Hz, 2H, 3-COCH₂), 3.07 (t, J=7 Hz, 2H, 1-COCH₂), and 8.23 (d, J=6 Hz, 1H, C⁶-H); UV_{max} (CH₃CN) 262 nm ($\epsilon=1.051 \times 10^4$).

1,3-bis-Valeryl-5-fluorouracil (16)

Extraction of the residue with hot low-boiling petroleum ether gave a cloudy solution which cleared upon cooling to 0 °C and produced a brown resinous sediment. The mixture was allowed to warm to room temperature, and the clear solution was decanted from the sediment. The solution was cooled again to 0 °C and crystallization gave 2.24 g of **16** (75%): mp 47-8 °C; IR (KBr) 1685, 1710, 1735, and 1795 cm⁻¹ (C=O); ¹H NMR (CDCl₃) δ 0.95 (t, J=7 Hz, 6H, 3-CH₃ and 1-CH₃),

1.3-1.8 (m, 8H, 3-COCH₂CH₂CH₂ and 1-COCH₂CH₂CH₂), 2.83 (t, J=7 Hz, 2H, 3-COCH₂), 3.08 (t, J=7 Hz, 2H, 1-COCH₂), and 8.22 (d, J=7 Hz, 1H, C⁶-H); UV_{max} (CH₃CN) 262 nm ($\epsilon=1.071 \times 10^4$).

Anal. Calc. for C₁₄H₁₉FN₂O₄: C, 56.37; H, 6.42; N, 9.39.
Found: C, 56.29; H, 6.48; N, 9.33.

Lipid solubility

Lipid solubilities were determined using isopropyl myristate (IPM), a commercial vehicle used in cosmetics and topical medicinals,⁹⁴ as the lipid solvent. The use of IPM as a model lipophilic vehicle in skin penetration studies is well established.^{77,95}

Three suspensions of each derivative were stirred at 22±1 °C for 48 hours. The suspensions were filtered through 0.45 µm nylon filters, and the saturated solutions were diluted in acetonitrile and analyzed by UV spectroscopy. Solubilities were calculated using Beer's Law:

$$A = \epsilon \cdot C \cdot d \quad (1)$$

where A is the absorbance, ϵ is the molar absorptivity, C is the concentration in mol/L, and d is the path length of the cuvette in cm. Molar absorptivities were predetermined in triplicate in acetonitrile at 262 nm.

Aqueous Solubility

Because of the chemical instability of the 1,3-bis-acyl derivatives, direct measurement of aqueous solubilities for

these prodrugs was not attempted. A comparison of the direct and partitioning-based methods for determining aqueous solubility was presented in Chapter 2 for the 1-alkyloxy-carbonyl derivatives.

Partition Coefficients

The partitioning-based method for determining aqueous solubility utilized the saturated IPM solutions from the lipid solubility study. For most compounds, equal volumes (1 mL) of saturated IPM solution and 0.05 M acetate buffer (pH=4.0) were used. The use of equal or near-equal phase volumes is known to facilitate rapid equilibrium.⁹⁶ The two phases were mixed thoroughly for ten seconds and allowed to separate for 60 seconds. A preliminary study showed that there was virtually no difference in partition coefficient (PC) values when partitioning was carried out for 10, 20, or 30 seconds (see Chapter 3). The IPM layers were diluted in acetonitrile and analyzed by UV spectroscopy. The IPM-buffer partition coefficients were calculated as follows:

$$PC = A_{\text{after}} / (A_{\text{before}} - A_{\text{after}}) \cdot V_{\text{AQ}} / V_{\text{IPM}} \quad (2)$$

where A_{after} is the absorbance from the IPM layer after partitioning, A_{before} is the absorbance from the IPM layer before partitioning, V_{AQ} is the volume of the aqueous phase, and V_{IPM} is the volume of the IPM phase. Estimated aqueous solubilities (S_{AQ}) were calculated from the IPM solubility

(S_{IPM}) and the partition coefficient:

$$S_{AQ} = S_{IPM}/PC \quad (3)$$

Partitioning was carried out in triplicate for a fixed volume ratio for each derivative. For those compounds with large differences in solubility in one phase relative to the other, volume ratios (IPM:buffer) other than 1:1 were necessary, but the ratio never exceeded 10:1 or 1:10.

Hydrolysis Kinetics

Hydrolysis rates have previously been reported for several members of this homologous series.²⁶ In the present study, hydrolysis rates for the conversion of 1,3-bis-acetyl-5FU (**13**) to 3-acetyl-5FU were determined at 32 °C in 0.05 M phosphate buffer (pH=7.1, I=0.12) and in the same buffer with 0.11% formaldehyde (3.6x10⁻² M). The rate in the presence of formaldehyde was determined for comparison with the rate in plain buffer since formaldehyde was used as a preservative in the diffusion cell experiments described in the following section.

The hydrolyses were followed by UV spectroscopy at 266 nm where the absorbance decrease accompanying conversion of the 1,3-bis-acetyl derivatives to 3-acetyl derivatives was maximized. Hydrolysis was initiated by adding 60 µL of a stock solution of the derivative in acetonitrile to 3 mL of buffer prewarmed to 32 °C in a thermostated quartz cuvette to give final concentrations of ~1.8x10⁻⁴ M. Absorbances were

recorded at appropriate intervals and pseudo-first-order rate constants were determined from the expression:

$$\ln(A_t - A_{\infty}) = \ln(A_0 - A_{\infty}) - kt \quad (4)$$

where A_t is the absorbance at some time t , A_{∞} is the absorbance at $t = \infty$, A_0 is the absorbance at $t = 0$, k is the pseudo-first-order rate constant, and t is the time. The hydrolyses were sufficiently fast and the 3-acyl products were stable enough to allow experimental determination of A_{∞} . The slopes, $-k$, of linear plots of $\ln(A_t - A_{\infty})$ versus time were determined by linear regression. The half-lives ($t_{1/2}$) were calculated from

$$t_{1/2} = 0.693/k \quad (5)$$

Each hydrolysis reaction was run in triplicate and was followed for a minimum of three half-lives. The correlation coefficients were ≥ 0.999 .

Skin Penetration Studies

Diffusion cell experiments were performed to measure the transdermal delivery of 5FU and the 5FU prodrugs. Franz-type diffusion cells from Crown Glass in Somerville, NJ with 4.9 cm² donor surface area and 20 mL receptor phase volume were used for this purpose. The full-thickness skins were obtained from female hairless mice (SKH-hr-1) from Temple University Skin and Cancer Hospital.

The mice were killed by cervical dislocation, their skins were removed immediately by blunt dissection, and

dorsal sections were mounted in the diffusion cells. The dermal sides of the skins were placed in contact with receptor phase which contained 0.05 M phosphate buffer (pH=7.1, I=0.12) with 0.11% formaldehyde as a preservative. The effectiveness of formaldehyde for this purpose has recently been documented.⁸⁹ The receptor phases were stirred continuously and kept at constant temperature (32 °C) by a circulating water bath. A preapplication period of 48 hours was established to uniformly condition the skins and to remove water-soluble UV-absorbing materials. The receptor phases were changed three times during this period, and control experiments from earlier studies have shown that this procedure effectively removes those materials.⁹⁷ The epidermal sides of the skins were exposed to the air and were left untreated during this period.

After the preapplication period, 0.5 mL aliquots from suspensions of the prodrugs in IPM were applied to the epidermal sides of the skins. The IPM suspensions were stirred at 22 ± 1 °C for 48 hours prior to application to ensure that saturation was attained. Total concentrations of the IPM suspensions ranged from 0.5 M to 2.0 M with enough excess solid present to maintain saturation for the duration of the application period (see below). Each drug-vehicle combination was run in triplicate.

Samples were taken from the receptor phases at 4, 8, 12, 21, 24, 27, 30, 33, 36, 45, and 48 hours after donor phase application. The receptor phases were changed following

removal of each sample so that "sink" conditions were maintained. Samples were analyzed for total 5FU species that had diffused by UV spectroscopy ($\epsilon=7.13 \times 10^3$ at 266 nm) after allowing at least 72 hours for complete prodrug hydrolysis. Cumulative amounts of total 5FU species that diffused (μmol) were plotted against time (h), and the slopes of the linear, "steady-state" regions were calculated using linear regression. The slopes, when divided by 4.9 (the area of the donor surface in cm^2), gave the "steady-state" fluxes ($\mu\text{mol}/\text{cm}^2/\text{h}$). Because of the rapid chemical hydrolysis of the 1,3-bis-acyl derivatives, no attempt was made to analyze the receptor phases for prodrug content.

Donor phases were changed every twelve hours and were set aside for ^1H NMR analysis. Stability of the prodrugs in IPM was determined from the chemical shift of $\text{C}^6\text{-H}$. In dimethylsulfoxide- d_6 , the $\text{C}^6\text{-H}$ signal for 5FU appears at $\delta=7.73$ ppm, and for the 3-acyl derivatives, it appears at $\delta=7.90$. For each of the 1,3-bis-acyl derivatives, the same signal in dimethylsulfoxide- d_6 appears at $\delta>8.40$ ppm. Since this area of the spectrum is free from interference by IPM absorbances, the three signals can be identified and quantified if necessary.

Following removal of the donor phases after the 48-hour application period, the epidermal sides of the skins were washed three times with 5 mL portions of methanol to remove all remnants of prodrug and vehicle from the skin surfaces. This was accomplished quickly (<3 min) to minimize contact

time between the skins and methanol. The receptor phases were changed again, and the dermal sides were kept in contact with the fresh buffer for 23 hours while the epidermal sides were again left exposed to the air. After this "leaching" period, another sample was taken from each cell to measure the skin accumulation of total 5FU species.

Second applications to the epidermal sides of the skins were made after the "leaching" period with a standard drug-vehicle suspension. Theophylline in propylene glycol (0.4 M) was applied to assess the damage to the skins from application of the initial drug-vehicle combinations. Samples were taken at 1, 2, 3, 5, 7, 9, and 11 or 12 hours after application. The samples were analyzed for theophylline by UV spectroscopy ($\epsilon=1.02 \times 10^4$ at 271 nm) and second application fluxes were determined as described above.

Results and Discussion

Synthesis and Structure Determination

The known 1,3-bis-acyl-5FU derivatives have melting points²⁵⁻²⁶ and ^1H NMR spectra²⁵ in agreement with those reported in the literature. The structures of novel compounds were assigned by comparison of their ^1H NMR spectra with those of the known homologs. Elemental microanalyses were obtained for the novel compounds and were within acceptable limits ($\pm 0.4\%$).

Structural analysis of the 1,3-bis-acetyl derivatives was helpful in resolving the apparent discrepancy between acidity and reactivity at the two attachment sites. In Chapter 1, it was noted that the monoanion of 5FU is actually a mixture of N^1 - and N^3 -anions,³⁰ and that a comparison of ionization constants for 5FU derivatives identically substituted at the N^1 - and N^3 -positions suggests that the N^3 -position is more acidic.^{22,30} This is supported by spectral studies in aqueous media (IR,¹⁰⁶ UV,¹⁰⁶⁻⁷ and ^1H NMR¹⁰⁸) which showed that for the 5FU monoanion, the N^3 -anion is the dominant form. Formation of the monoanion, however, leads exclusively to N^1 -acylated products (Chapter 2 and Chapter 3). In addition, the 1,3-bis-acetyl derivatives undergo rapid N^1 -deacylation during hydrolysis.²⁶ Ordinarily, this would imply that the N^1 -anion is more stable than the N^3 -anion and that the N^1 -position is more acidic.

The structure of 1,3-bis-acetyl-5FU (**13**) from single crystal X-ray analysis is shown in Figure 4-1. The carbonyl bond in the N^1 -acetyl group is positioned cis to the C^6 -H bond as it is in 1-acetyl-5FU (**7**). As expected, the chemical shifts of C^6 -H in chloroform-d show the same anisotropic effect for compounds **7** and **13**. The N^3 -acetyl group, however, is shown to be nearly perpendicular to the plane of the 5FU ring. Apparently, it is being forced out of plane by the carbonyl groups at the C^2 - and C^4 -positions.

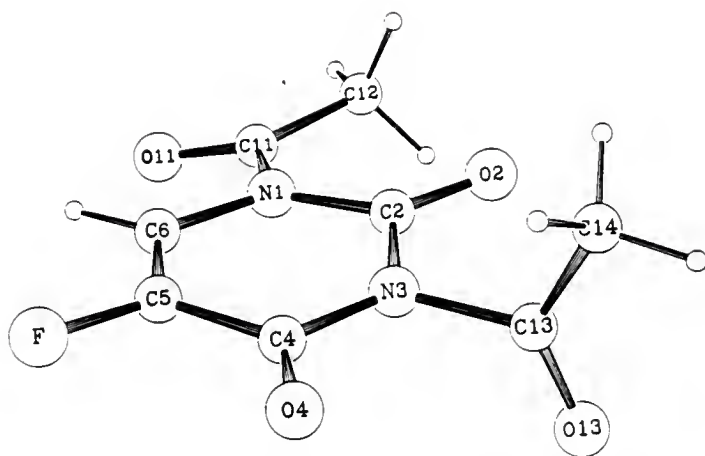


Figure 4-1. X-ray structure of 1,3-bis-acetyl-5FU.
Source: Hoffman-La Roche, Nutley, NJ.

The steric constraints at the N³-position may explain the lower than expected reactivity at this site. The C²- and C⁴-carbonyl groups make acylation more difficult by restricting the orientations available to the relatively bulky acylating agents as they interact with the 5FU anion. Likewise, slower hydrolysis rates are due to partial negative charges on oxygen at C²=O and C⁴=O which impede the approach of hydroxide ions to the N³-acyl carbonyl group when it is perpendicular to the plane of the 5FU ring. Thus, although the N³-anion is a better leaving group than the N¹-anion, steric hindrance prevents more rapid hydrolysis of the N³-acyl group than the N¹-acyl group in the 1,3-bis-acyl series.

Infrared (IR) spectra for the 1,3-bis-acyl and 3-acyl derivatives are also consistent with the conformation depicted in Figure 4-1. The carbonyl stretching frequency for the N³-acyl group is 25 to 50 cm⁻¹ higher than those associated with the 5FU ring or N¹-acyl groups. The perpendicular orientation of the N³-acyl group apparently prevents electron delocalization from the ring to the carbonyl bond which means that the inductive effect of the ring predominates, and the carbonyl stretching frequency is increased.¹⁰⁵

Solubility

Lipid (S_{IPM}) and aqueous (S_{AQ}) solubilities for the 1,3-bis-acyl derivatives are presented in Table 4-2 along with their melting points. The downward trend in melting point and upward trend in lipid solubility with increasing chain length is pronounced in these diacylated compounds.

Aqueous solubilities were only determined for the first two derivatives, and both are at least an order of magnitude less than 5FU. The longer chain derivatives exhibit such high lipid solubilities compared to their expected aqueous solubilities that partitioning could not be done without using unreasonable IPM-Buffer ratios.

Hydrolysis Kinetics

Hydrolysis of 1,3-bis-acetyl-5FU (**13**) to 3-acetyl-5FU was followed by UV spectroscopy in 0.05 M phosphate buffer (pH=7.1, $I=0.12$) at 32 °C with and without 0.11% formaldehyde (3.6×10^{-2} M). Disappearance of compound **13**, indicated by $\ln(A_t - A_\infty)$, is plotted versus time (min) in Figure 4-2. The linearity of the plots suggests that hydrolysis of **13** follows first-order kinetics in the presence and absence of formaldehyde. Pseudo-first-order rate constants (k) and half-lives ($t_{1/2}$) from the linear plots are presented in Table 4-3. The much greater stability of the product

Table 4-2. Melting points (MP), lipid solubilities (S_{IPM}), and aqueous solubilities (S_{AQ}) for 1,3-bis-acyl derivatives.

Compound	MP (°C)	S_{IPM}^a (mM)	S_{AQ}^b (mM)	S_{AQ}^c (mM)
5FU	280-2	0.049	96	-
13	112-3	26	-	9.0
14	100-1	72	-	3.3
15	48-9	625	-	-
16	47-8	1180	-	-

^aStandard deviation from the means were within $\pm 4\%$ for IPM solubilities.

^bSolubility determined by direct method.

^cStandard deviations from the mean were within $\pm 4\%$ for experimental values used to calculate partition coefficients and estimated aqueous solubilities.

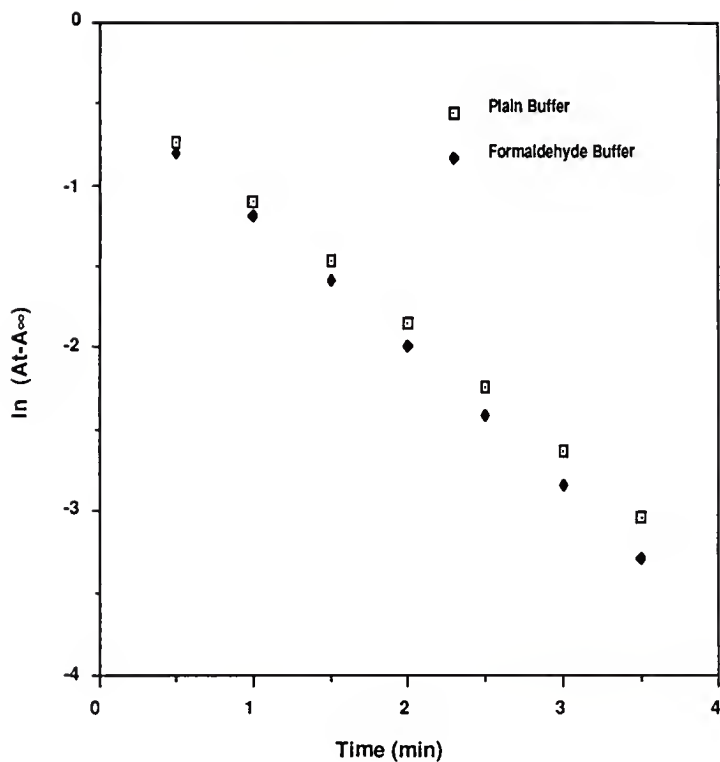


Figure 4-2. Plots of $\ln(A_t - A_\infty)$ versus time (min) for hydrolysis of 1,3-bis-acetyl-5FU in 0.05 M phosphate buffer (pH=7.1, $I=0.12$) with and without formaldehyde at 32 °C.

Table 4-3. Pseudo-first-order rate constants (k) and half-lives ($t_{1/2}$) for hydrolysis of 1,3-bis-acetyl-5FU in 0.05 M phosphate buffer (pH=7.1, $I=0.12$) with and without formaldehyde at 32 °C.

Compound	Formaldehyde (M)	$k (\pm SD)^a$ (min ⁻¹)	$t_{1/2}$ (min)
13	0	0.802(0.010)	0.86
13	3.6×10^{-2}	0.868(0.039)	0.80

^aMean $\pm$ standard deviation for $n=3$ values.

3-acyl derivatives compared to the 1,3-bis-acyl derivatives allows the rates of hydrolysis for the N¹-acyl groups to be followed for three to four half-lives before detectable hydrolysis of the N³-acyl groups is observed. The hydrolysis of the 3-acyl derivatives will be discussed in Chapter 5.

Hydrolysis of compound **13** is clearly faster in the presence of formaldehyde indicating that general base catalysis by formaldehyde hydrate may be involved in this series as well (see Chapter 3). Complete pH-rate profiles for selected acyl derivatives of 5FU can be found in the work of Buur and Bundgaard.²⁶

Skin Penetration

Skin penetration data from the diffusion cells are plotted as cumulative amount of total 5FU species that diffused (μmol) versus time (h). In Figure 4-3, results for 1,3-bis-acetyl-5FU (**13**) and 1,3-bis-propionyl-5FU (**14**) are compared to 5FU itself. In Figure 4-4, results for 1,3-bis-butyryl-5FU (**15**) and 1,3-bis-valeryl-5FU (**16**) are compared to 5FU. Error bars correspond to the standard deviation from the mean for n=3 values.

Fluxes (J), lag times (t_L), and skin accumulation (SA) values for each compound are reported in Table 4-4. The highest flux obtained for the 1,3-bis-acyl series is for 1,3-bis-acetyl-5FU (**13**) with nearly an order of magnitude improvement over 5FU. Overall, the 1,3-bis-acyl derivatives

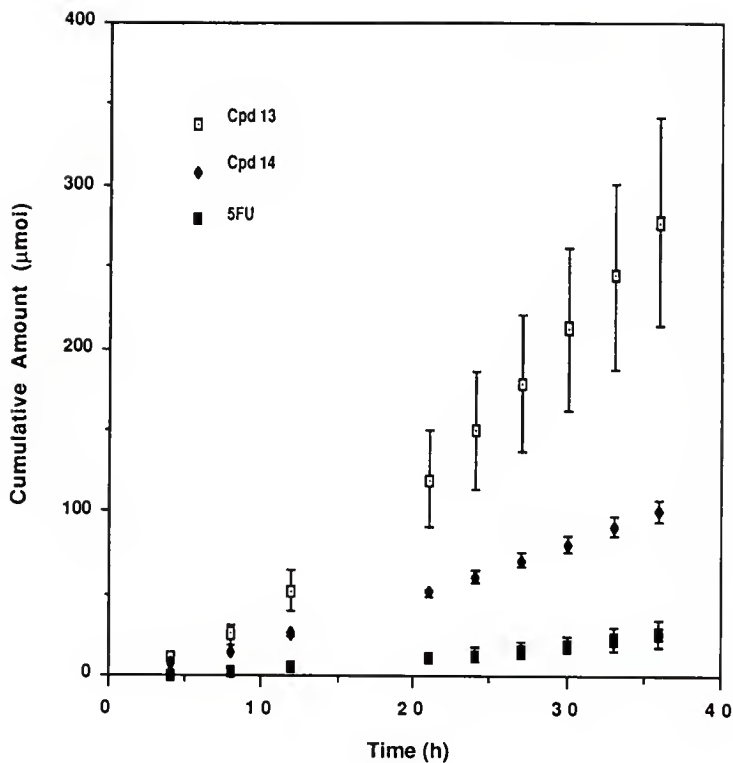


Figure 4-3. Plots of cumulative amount of total 5FU species that diffused (μmol) versus time (h) for compounds 13, 14, and 5FU.

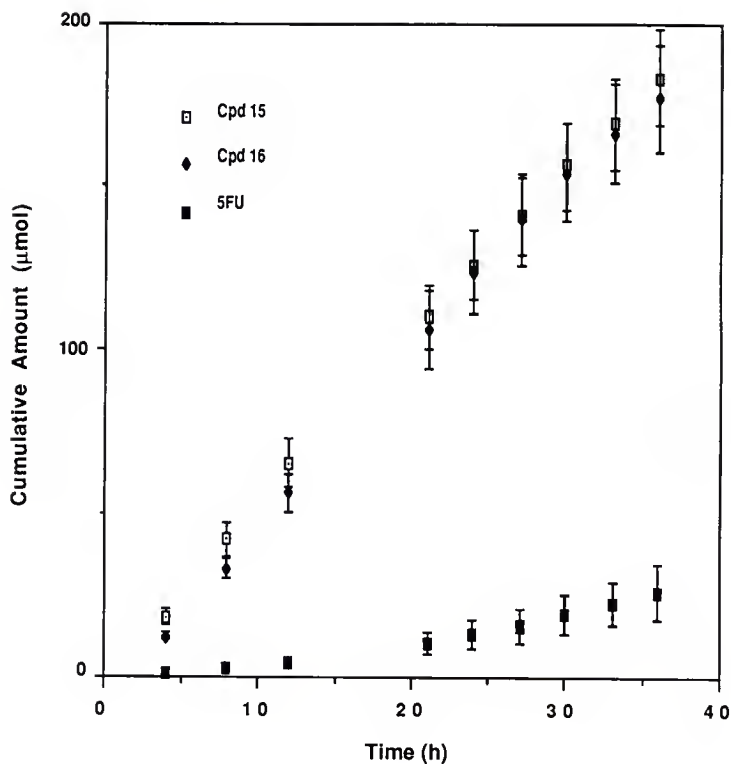


Figure 4-4. Plots of cumulative amount of total 5FU species that diffused (μmol) versus time (h) for compounds 15, 16, and 5FU.

Table 4-4. Fluxes (J), lag times (t_L), and skin accumulation (SA) values for 1,3-bis-acyl derivatives.

Compound	J ($\pm$ SD) ^a ($\mu\text{mol}/\text{cm}^2/\text{h}$)	t_L (h)	SA ($\pm$ SD) ^a (μmol)
5FU	0.24 (0.09)	13	3.7 (0.9)
13	2.2 (0.5)	11	10 (2)
14	0.69 (0.06)	6.2	4.5 (1.5)
15	0.98 (0.06)	-2.3	12 (2)
16	0.95 (0.05)	-2.8	8.8 (1.8)

^aMean $\pm$ standard deviation for n=3 values.

Table 4-5. Second application fluxes (J) and lag times (t_L) for 1,3-bis-acyl derivatives.

Compound	$J^a(\pm SD)^b$ ($\mu\text{mol}/\text{cm}^2/\text{h}$)	$t_L(\text{h})$
5FU	1.2 (0.2)	1.2
13	1.6 (0.1)	0.7
14	1.6 (0.3)	0.7
15	1.1 (0.1)	0.9
16	0.87 (0.14)	0.8

^aFlux of 0.4 M theopylline from propylene glycol.

^bMean $\pm$ standard deviation for n=3 values.

are much less effective at delivering 5FU transdermally than the 1-alkyloxycarbonyl and 1-acyl series. The results for this highly lipophilic, but poorly aqueous-soluble series demonstrate the importance of biphasic solubility for maximizing skin penetration.

The negative lag times observed for 1,3-bis-butyryl-5FU (**15**) and 1,3-bis-valeryl-5FU (**16**) may have been caused by a change in the physical state of the donor phase. A large amount of solid was required in formulating the donor phases for compounds **15** and **16** due to their high solubility in IPM. After a few hours of skin contact at 32 °C, it became difficult to determine if excess solid from these low-melting derivatives was still present in the donor phases since their appearance was more like a gel than a suspension.

Skin accumulation values are greater for the 1,3-bis-acyl series than for 5FU, but the improvement is small. The best derivatives, compounds **13**, **15**, and **16**, have skin accumulation values less than three times higher than 5FU.

Second application fluxes and lag times are reported in Table 4-5. Skin penetration by theophylline from propylene glycol, the standard drug-vehicle combination, after treatment with the 1,3-bis-acyl derivatives is similar to that following treatment with 5FU.

The stability of the prodrugs in the IPM formulations was assessed by ^1H NMR analysis of the donor phases. After a minimum of five days from the time the suspensions were prepared until their ^1H NMR spectra were recorded, including

at least twelve hours during which the formulations were in contact with the skins, the 1,3-bis-acyl derivatives were found to be intact with no evidence of formation of the corresponding 3-acyl derivatives or 5FU.

Summary

The 1,3-bis-acyl derivatives of 5FU exhibited decreased melting points and greatly increased lipid solubilities when compared to 5FU. Aqueous solubility was at best an order of magnitude less than 5FU and was highest for 1,3-bis-acetyl-5FU (**13**). Flux was also maximized for compound **13** which again shows the importance of aqueous solubility for optimizing skin penetration within a homologous series of more lipid-soluble prodrugs. Biphasic solubility data appears to be useful for series to series comparisons as well. The highly lipophilic 1,3-bis-acyl derivatives were less effective at delivering 5FU transdermally than the less lipid-soluble, but more aqueous-soluble series such as the 1-alkyloxycarbonyl and 1-acyl series. The partitioning-based method for determining aqueous solubilities of chemically unstable prodrugs was used to estimate the aqueous solubilities of two of the 1,3-bis-acyl derivatives since this could not be accomplished by other methods. The difference in the stabilities of the N¹- and N³-acyl groups of the 1,3-bis-acyl derivatives has been rationalized based on an X-ray crystallography study which showed that the N³-acyl

group is oriented perpendicular to the plane of the 5FU ring and is sterically and electronically hindered from nucleophilic attack.

CHAPTER 5 3-ACYL DERIVATIVES

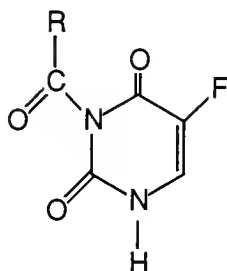
Introduction

The 3-acyl derivatives of 5FU are more stable chemically than either the 1-acyl or 1,3-bis-acyl derivatives.²⁶ Enzymatic hydrolysis proceeds at a faster rate than chemical hydrolysis, and this difference is more pronounced for the short-chain derivatives of the 3-acyl series²⁶ some of which may be useful as prodrugs for dermal delivery.

The 3-acyl derivatives were chosen for evaluation because of their chemical stability and for comparison with the 1-acyl series. It was known that N¹- and N³-substituted derivatives have different physical-chemical properties,^{22,29-30} and it was likely that they would exhibit differences in skin penetration as well.

Four straight chain 3-acyl derivatives were selected for study. The derivatives and their structures are presented in Table 5-1.

Table 5-1. Structures of 3-acyl derivatives.



Compound	R
3-acetyl-5FU (17)	-CH ₃
3-propionyl-5FU (18)	-CH ₂ CH ₃
3-butyryl-5FU (19)	-(CH ₂) ₂ CH ₃
3-valeryl-5FU (20)	-(CH ₂) ₃ CH ₃

Materials and Methods

Synthesis

Melting points (mp) were determined with a Thomas-Hoover capillary melting point apparatus and are uncorrected. Elemental microanalyses were obtained for all novel compounds through Atlantic Microlab, Incorporated in Norcross, Georgia. Proton nuclear magnetic resonance (^1H NMR) spectra were obtained at 90 MHz on a Varian EM-390 spectrometer. Chemical shifts (δ) are reported in parts per million (ppm) from the internal standard, tetramethylsilane (TMS). Coupling constants (J) are expressed in cycles per second (Hz). Infrared (IR) spectra were recorded with a Perkin-Elmer 1420 spectrophotometer and absorbances are reported in cm^{-1} . Ultraviolet (UV) spectra were obtained with a Cary 210 or Shimadzu UV-265 spectrophotometer. Maximum absorbances are reported in nm along with the molar absorptivities (ϵ) in L/mol. Thin layer chromatography (TLC) was run on Brinkman Polygram Sil G/UV254 0.25 mm silica gel.

3-Acyl-5-fluorouracil (general procedure)

To a solution of an appropriate 1,3-bis-acyl derivative (0.01 mol) in ether (60-650 mL) at 0 °C was added 0.73 g of tert-butylamine (0.01 mol) in ether (25 mL) dropwise over ten minutes with stirring. The solution was stirred for a total of 30 minutes at 0 °C. The ether solution was evaporated

under reduced pressure at room temperature until the volume was reduced to 25-50 mL. The solution was cooled again to 0 °C until crystallization occurred.

3-Acetyl-5-fluorouracil (17)

Crystallization gave 1.22 g of **17** (71%): mp 115-7 °C dec (lit.²⁵ mp 114-7 °C); IR (KBr) 1650, 1685, 1720, and 1805 cm^{-1} (C=O); ^1H NMR (CDCl_3) δ 2.58 (s, 3H, CH_3) and 7.23 (bs, 1H, $\text{C}^6\text{-H}$); UV_{max} (CH_3CN) 267 nm ($\epsilon=6.57 \times 10^3$).

3-Propionyl-5-fluorouracil (18)

Crystallization gave 0.89 g of **18** (48%): mp 102-3 °C dec (lit.²⁵ mp 99-102 °C); IR (KBr) 1655, 1680, 1730, and 1815 cm^{-1} (C=O); ^1H NMR (CDCl_3) δ 1.27 (t, $J=7$ Hz, 3H, CH_3), 2.86 (q, $J=7$ Hz, 2H, COCH_2), and 7.26 (bs, 1H, $\text{C}^6\text{-H}$); UV_{max} (CH_3CN) 267 nm ($\epsilon=6.66 \times 10^3$).

3-Butyryl-5-fluorouracil (19)

Crystallization gave 1.22 g of **19** (61%): mp 111-2 °C (lit.²⁶ mp 132-4 °C); IR (KBr) 1660, 1730, and 1810 cm^{-1} (C=O); ^1H NMR (CDCl_3) δ 1.02 (t, $J=7$ Hz, 3H, CH_3), 1.6-2.0 (m, 2H, COCH_2CH_2), 2.82 (t, $J=7$ Hz, 2H, COCH_2), and 7.26 (bs, 1H, $\text{C}^6\text{-H}$); UV_{max} (CH_3CN) 267 nm ($\epsilon=6.55 \times 10^3$).

Anal. Calc. for $\text{C}_8\text{H}_9\text{FN}_2\text{O}_3$: C, 48.00; H, 4.53; N, 14.00.
Found: C, 48.08; H, 4.55; N, 13.93.

3-Valeryl-5-fluorouracil (20)

Crystallization gave 1.54 g of **20** (72%): mp 110-1 °C dec; IR (KBr) 1665, 1730, and 1815 cm^{-1} (C=O); ^1H NMR (CDCl_3) δ 0.97 (t, $J=7$ Hz, 3H, CH_3), 1.3-1.9 (m, 4H, $\text{COCH}_2\text{CH}_2\text{CH}_2$),

2.84 (t, $J=7$ Hz, 2H, COCH_2), and 7.23 (bs, 1H, $\text{C}^6\text{-H}$); UV_{max} (CH_3CN) 267 nm ($\epsilon=6.68 \times 10^3$).

Anal. Calc. for $\text{C}_9\text{H}_{11}\text{FN}_2\text{O}_3$: C, 50.47; H, 5.18; N, 13.08.
Found: C, 50.73; H, 5.24; N, 12.95.

Lipid solubility

Lipid solubilities were determined using isopropyl myristate (IPM), a commercial vehicle used in cosmetics and topical medicinals,⁹⁴ as the lipid solvent. The use of IPM as a model lipophilic vehicle in skin penetration studies is well established.^{77,95}

Three suspensions of each derivative were stirred at 22 ± 1 °C for 48 hours. The suspensions were filtered through $0.45 \mu\text{m}$ nylon filters, and the saturated solutions were diluted in acetonitrile and analyzed by UV spectroscopy. Solubilities were calculated using Beer's Law:

$$A = \epsilon \cdot C \cdot d \quad (1)$$

where A is the absorbance, ϵ is the molar absorptivity, C is the concentration in mol/L, and d is the path length of the cuvette in cm. Molar absorptivities were predetermined in triplicate in acetonitrile at 267 nm.

Aqueous Solubility

For direct measurement of aqueous solubilities, two or more suspensions of each derivative were vigorously stirred in 0.05 M acetate buffer (pH=4.0) at 22 ± 1 °C for 60 minutes.

The suspensions were filtered through 0.45 μm nylon filters, and the saturated solutions were diluted in acetonitrile and analyzed by UV spectroscopy. Solubilities were calculated using Beer's Law as previously described.

Partition Coefficients

The partitioning-based method for determining aqueous solubility utilized the saturated IPM solutions from the lipid solubility study. For most compounds, equal volumes (1 mL) of saturated IPM solution and 0.05 M acetate buffer (pH=4.0) were used. The use of equal or near-equal phase volumes is known to facilitate rapid equilibrium.⁹⁶ The two phases were mixed thoroughly for ten seconds and allowed to separate for 60 seconds. A preliminary study showed that there was virtually no difference in partition coefficient (PC) values when partitioning was carried out for 10, 20, or 30 seconds (see Chapter 3). The IPM layers were diluted in acetonitrile and analyzed by UV spectroscopy. The IPM-buffer partition coefficients were calculated as follows:

$$\text{PC} = A_{\text{after}} / (A_{\text{before}} - A_{\text{after}}) \cdot V_{\text{AQ}} / V_{\text{IPM}} \quad (2)$$

where A_{after} is the absorbance from the IPM layer after partitioning, A_{before} is the absorbance from the IPM layer before partitioning, V_{AQ} is the volume of the aqueous phase, and V_{IPM} is the volume of the IPM phase. Estimated aqueous solubilities (S_{AQ}) were calculated from the IPM solubility

(S_{IPM}) and the partition coefficient:

$$S_{AQ} = S_{IPM}/PC \quad (3)$$

Partitioning was carried out in triplicate for a fixed volume ratio for each derivative. For those compounds with large differences in solubility in one phase relative to the other, volume ratios (IPM:buffer) other than 1:1 were necessary, but the ratio never exceeded 10:1 or 1:10.

Hydrolysis Kinetics

Hydrolysis rates have previously been reported for several members of this homologous series.²⁶ In the present study, hydrolysis rates were determined at 32 °C for 3-acetyl-5FU (**17**) in 0.05 M phosphate buffer (pH=7.1, $I=0.12$) and in the same buffer with 0.11% formaldehyde (3.6×10^{-2} M). The rate in the presence of formaldehyde was determined for comparison with the rate in plain buffer since formaldehyde was used as a preservative in the diffusion cell experiments described in the following section. Two other concentrations of formaldehyde were studied (3.6×10^{-3} M and 3.6×10^{-4} M) when it was discovered that products in addition to 5FU were being formed during hydrolysis of compound **17**.

The hydrolyses were followed by high performance liquid chromatography (HPLC). The HPLC system consisted of a Beckman model 110A pump with a model 153 UV detector, a Rheodyne 7125 injector with a 20 μ m loop, and a Hewlett-Packard 3392A integrator. The column was a Lichrosorb RP-8

10 μm reversed-phase column, 250 mm x 4.6 mm (inside diameter). The mobile phase contained 10% methanol and 90% 0.025 M acetate buffer (pH=5.0) and was run at 1.0 mL/min. The column effluent was monitored at 254 nm, and quantitation was based on peak areas. Standards chromatographed under the same conditions were used for calibration.

Hydrolysis was initiated by adding 0.4 mL of a stock solution of compound **17** in acetonitrile to 25 mL of buffer prewarmed to 32 $^{\circ}\text{C}$ in a constant temperature water bath to give final concentrations of $\sim 1.8 \times 10^{-4}$ M. Aliquots were removed at appropriate intervals and chromatographed immediately. Pseudo-first-order rate constants were determined from the expression:

$$\ln(C_t) = \ln(C_0) - kt \quad (4)$$

where C_t is the concentration at some time t , C_0 is the concentration at $t=0$, k is the pseudo-first-order rate constant, and t is the time. The slopes, $-k$, of linear plots of $\ln(C_t)$ versus t were determined by linear regression. The half-lives ($t_{1/2}$) were calculated from

$$t_{1/2} = 0.693/k \quad (5)$$

The hydrolyses of compounds **17** and **18** (3-propionyl-5FU) in buffer alone were followed by UV spectroscopy at 300 nm where the absorbance decrease accompanying hydrolysis was maximized. Hydrolysis was initiated by adding 60 μL of a stock solution of the derivative in acetonitrile to 3 mL of buffer prewarmed to 32 $^{\circ}\text{C}$ in a thermostatted quartz cuvette to give final concentrations of $\sim 1.8 \times 10^{-4}$ M. Absorbances were

recorded at appropriate intervals and pseudo-first-order rate constants were determined from the expression:

$$\ln(A_t - A_{\infty}) = \ln(A_0 - A_{\infty}) - kt \quad (6)$$

where A_t is the absorbance at some time t , A_{∞} is the absorbance at $t = \infty$, A_0 is the absorbance at $t = 0$, k is the pseudo-first-order rate constant, and t is the time. The hydrolyses were sufficiently fast to allow experimental determination of A_{∞} . The slopes, $-k$, of linear plots of $\ln(A_t - A_{\infty})$ versus time were determined by linear regression. The half-lives ($t_{1/2}$) were calculated from equation (5).

The hydrolysis reactions were run in duplicate or triplicate and were followed for a minimum of three half-lives. The correlation coefficients for all reported rate constants were ≥ 0.999 except where noted.

Skin Penetration Studies

Diffusion cell experiments were performed to measure the transdermal delivery of 5FU and the 5FU prodrugs. Franz-type diffusion cells from Crown Glass in Somerville, NJ with 4.9 cm² donor surface area and 20 mL receptor phase volume were used for this purpose. The full-thickness skins were obtained from female hairless mice (SKH-hr-1) from Temple University Skin and Cancer Hospital.

The mice were killed by cervical dislocation, their skins were removed immediately by blunt dissection, and dorsal sections were mounted in the diffusion cells. The

dermal sides of the skins were placed in contact with receptor phase which contained 0.05 M phosphate buffer (pH=7.1, I=0.12) with 0.11% formaldehyde as a preservative. The effectiveness of formaldehyde for this purpose has recently been documented.⁸⁹ The receptor phases were stirred continuously and kept at constant temperature (32 °C) by a circulating water bath. A preapplication period of 48 hours was established to uniformly condition the skins and to remove water-soluble UV-absorbing materials. The receptor phases were changed three times during this period, and control experiments from earlier studies have shown that this procedure effectively removes those materials.⁹⁷ The epidermal sides of the skins were exposed to the air and were left untreated during this period.

After the preapplication period, 0.5 mL aliquots from suspensions of the prodrugs in IPM were applied to the epidermal sides of the skins. The IPM suspensions were stirred at 22 ± 1 °C for 48 hours prior to application to ensure that saturation was attained. Total concentrations of the IPM suspensions ranged from 0.4 M to 0.6 M with enough excess solid present to maintain saturation for the duration of the application period (see below). Each drug-vehicle combination was run in triplicate.

Samples were taken from the receptor phases at 4, 8, 12, 21, 24, 27, 30, 33, 36, 45, and 48 hours after donor phase application. The receptor phases were changed following removal of each sample so that "sink" conditions were

maintained. Samples were analyzed for total 5FU species that had diffused by UV spectroscopy ($\epsilon=7.13 \times 10^3$ at 266 nm) after allowing at least 72 hours for complete prodrug hydrolysis. Cumulative amounts of total 5FU species that diffused (μmol) were plotted against time (h), and the slopes of the linear, "steady-state" regions were calculated using linear regression. The slopes, when divided by 4.9 (the area of the donor surface in cm^2), gave the "steady-state" fluxes ($\mu\text{mol}/\text{cm}^2/\text{h}$). In a separate experiment, HPLC was used to determine intact prodrug content in the receptor phases at each sampling time. Mobile phase containing 18-50% methanol in 0.025 M acetate buffer (pH=5.0) was used with the system described earlier. Aliquots were removed and chromatographed immediately after the samples were taken. Prodrug fluxes were calculated in the same manner as fluxes for total 5FU.

Donor phases were changed every twelve hours and were set aside for ^1H NMR analysis. Stability of the prodrugs in IPM was determined from the chemical shift of $\text{C}^6\text{-H}$. In dimethylsulfoxide- d_6 , the $\text{C}^6\text{-H}$ signal for 5FU appears at $\delta=7.73$ ppm. For each of the 3-acyl derivatives, the same signal in dimethylsulfoxide- d_6 appears at $\delta=7.90$. Since this area of the spectrum is free from interference by IPM absorbances, the two signals can be identified and quantified if necessary.

Following removal of the donor phases after the 48-hour application period, the epidermal sides of the skins were washed three times with 5 mL portions of methanol to remove

all remnants of prodrug and vehicle from the skin surfaces. This was accomplished quickly (<3 min) to minimize contact time between the skins and methanol. The receptor phases were changed again, and the dermal sides were kept in contact with the fresh buffer for 23 hours while the epidermal sides were again left exposed to the air. After this "leaching" period, another sample was taken from each cell to measure the skin accumulation of total 5FU species.

Second applications to the epidermal sides of the skins were made after the "leaching" period with a standard drug-vehicle suspension. Theophylline in propylene glycol (0.4 M) was applied to assess the damage to the skins from application of the initial drug-vehicle combinations. Samples were taken at 1, 2, 3, 5, 7, 9, and 11 or 12 hours after application. The samples were analyzed for theophylline by UV spectroscopy ($\epsilon=1.02 \times 10^4$ at 271 nm) and second application fluxes were determined as described above.

Results and Discussion

Synthesis and Structure Determination

The known 3-acyl-5FU derivatives have melting points²⁵⁻²⁶ and ^1H NMR spectra²⁵ in agreement with those reported in the literature. The structures of novel compounds were assigned by comparison of their ^1H NMR spectra with those of the known

homologs. Elemental microanalyses were obtained for the novel compounds and were within acceptable limits ($\pm 0.4\%$).

Differences in the spectral properties between N^1 - and N^3 -acylated derivatives were discussed in detail in Chapter 2 and Chapter 3. The 3-acyl derivatives are synthesized by N^1 -deacylation of the 1,3-bis-acyl derivatives, and ^1H NMR and IR spectra confirm that it is the N^3 -acyl groups that are present in the products.

The melting points of the 3-acyl derivatives are rate dependent and exhibit an incomplete melt followed by resolidification and a complete melt at 40 to 100 °C above the initial thermal event suggesting thermal decomposition of the prodrugs. The literature melting points for 3-propionyl-5FU (**18**) are 99-102 °C²⁵ and 113-4 °C.²⁶ In the present study, the melting point for compound **18** was 102-3 °C when the temperature was raised by 2 °C per minute, but if the rate was increased to 5 °C per minute, the observed melting point was 111-3 °C.

The thermal decomposition of 3-acetyl-5FU (**17**) was studied by dissolving 50 mg of compound **17** in 10 mL of ethyl acetate in a beaker and heating gently to evaporate the solvent. The temperature was increased slowly until the residual solid had melted completely. The beaker was then removed from the heat to allow resolidification to occur.

The decomposition was followed by TLC on silica gel using ethyl acetate as the eluent. The TLC showed no decomposition until after the solvent had completely

evaporated at which time gradual disappearance of compound **17** ($R_f=0.43$) began with formation of both 1-acetyl-5FU ($R_f=0.62$) and 1,3-bis-acetyl-5FU ($R_f=0.66$) as well as 5FU ($R_f=0.30$). Upon resolidification of the melt, its ^1H NMR spectrum showed that compound **17** had been converted to 1-acetyl-5FU (~70%), 5FU (~30%) and a trace of 1,3-bis-acetyl-5FU. Percentages were calculated from integration of the corresponding $\text{C}^6\text{-H}$ signals.

Similar studies showed that melting 1,3-bis-acetyl-5FU alone produced 1-acetyl-5FU (~40%) and 1,3-bis-acetyl-5FU (~60%) while melting equivalent amounts of 1,3-bis-acetyl-5FU and 5FU produced 1-acetyl-5FU (~75%), 5FU (~20%), and 1,3-bis-acetyl-5FU (~5%).

Since rate and duration of heating were not controlled in these experiments, conclusions regarding the relative amounts of decomposition products formed can not be drawn. However, the data do suggest that thermal N^3 -deacylation occurs from both 3-acetyl-5FU and 1,3-bis-acetyl-5FU, and overall, there is a net loss of acetyl groups. Some of the N^3 -acetyl groups (or even N^1 -acetyl groups) could be lost to ketene formation as shown in Figure 5-1. There is also a net gain of N^1 -acetyl groups when 3-acetyl-5FU is heated alone or a mixture of 1,3-bis-acetyl-5FU and 5FU is heated. This indicates that a rearrangement has occurred. The TLC and ^1H NMR data suggest that the rearrangement is to some degree intermolecular since some 1,3-bis-acetyl-5FU is produced when 3-acetyl-5FU is heated alone, but an intramolecular

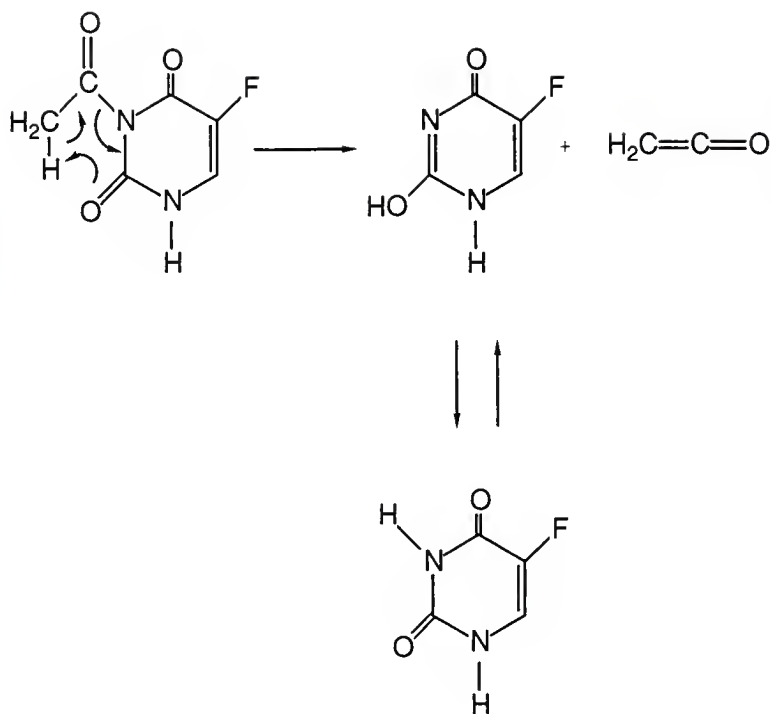


Figure 5-1. Possible scheme for thermal decomposition of 3-acetyl-5FU to 5FU.

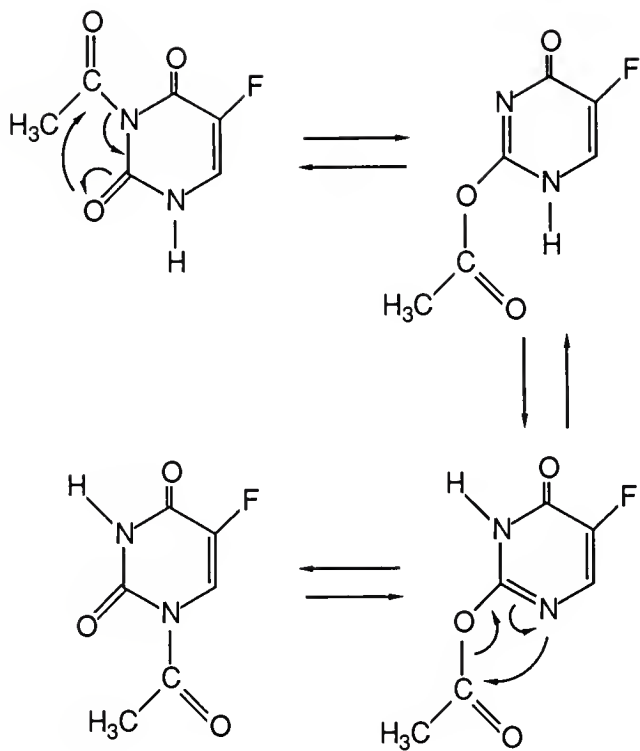


Figure 5-2. Possible scheme for thermal intramolecular rearrangement for 3-acetyl-5FU to 1-acetyl-5FU.

rearrangement from N³- to N¹-acetyl is possible in this case as well. In Figure 5-2, an intramolecular rearrangement scheme is presented in which a transient O²-acyl intermediate is proposed.

The existence of intramolecular 1,3 acyl migrations from both N to O and O to N has been documented.¹⁰⁹ Of the several possible mechanisms for the O to N thermal rearrangement, one that is preferred involves rate-determining nucleophilic attack by the nitrogen lone pair on the carbonyl carbon with partial C-O bond cleavage to relieve the strain associated with formation of a four-membered ring.¹¹⁰ This synchronous mechanism is depicted in Figure 5-2 for both the N to O and O to N rearrangements. The perpendicular orientation of the N³-acetyl group to the 5FU ring would facilitate a rearrangement of this type. All steps are shown as reversible since acyl or aroyl rearrangements are potentially reversible whether they are intramolecular or intermolecular.¹¹¹⁻³ It is worth noting that similar melting behavior to that seen for the 3-acyl derivatives in the present study was also observed for the O to N benzoyl migration in 6-phenanthridinone.¹¹³ Further discussion regarding the proposed O²-acyl intermediate will be presented in the hydrolysis kinetics section.

Solubility

Lipid (S_{IPM}) and aqueous (S_{AQ}) solubilities for the 3-acyl derivatives are presented in Table 5-2 along with their melting points. Lipid solubilities are greatly enhanced by making these derivatives, and solubility generally increases with increasing chain length. A change in this trend is observed for 3-valeryl-5FU (**20**). A similar downturn in lipid solubility was also seen for 1-butyryl-5FU in Chapter 3.

Because of the relative stability of the 3-acyl derivatives,²⁶ aqueous solubilities were determined by both the direct and partitioning-based methods. The results show that aqueous solubility is highest for 3-propionyl-5FU (**18**) and then decreases. Both compound **18** and 3-acetyl-5FU (**17**) exhibit aqueous solubilities greater than 5FU.

Aqueous solubilities determined by the partitioning-based method underestimated the direct solubilities by 32% for compound **18**, 37% for compound **17**, 57% for compound **19**, and overestimated the direct solubility by 10% for compound **20**. Relative aqueous solubility among members of the series is the same with either procedure, but agreement between the two methods is not as good as that seen for the 1-alkyloxycarbonyl derivatives.

Table 5-2. Melting points (MP), lipid solubilities (S_{IPM}), and aqueous solubilities (S_{AQ}) for 3-acyl derivatives.

Compound	MP (°C)	S _{IPM} ^a (mM)	S _{AQ} ^b (mM)	S _{AQ} ^c (mM)	S _{AQ} ^d (mM)
5FU	280-2	0.049	96	-	85
17	115-7	4.3	166	105	249
18	102-3	14	198	135	190
19	111-2	22	53	23	-
20	110-1	9.2	5.0	5.5	-

^aStandard deviations from the mean were within $\pm 3\%$ for IPM solubilities ($\pm 10\%$ for compound 19).

^bStandard deviations from the mean were within $\pm 8\%$ for aqueous solubilities determined by direct method

^cStandard deviations from the mean were within $\pm 5\%$ for experimental values used to calculate partition coefficients and estimated aqueous solubilities ($\pm 17\%$ for compound 19).

^dLiterature values from reference 26.

Table 5-3. Solubility ratios (SR), partition coefficients (PC), and hydrophobicity parameters (π) for 3-acyl derivatives.

Compound	SR ^a	log (SR)	π^b	PC ^c	log (PC)	π^d	log (PC) - log (SR)
17	0.026	-1.59	-	0.041	-1.39	-	0.20
18	0.072	-1.14	0.45	0.11	-0.97	0.42	0.17
19	0.42	-0.38	0.76	0.98	-0.01	0.96	0.37
20	1.9	0.27	0.65	1.7	0.22	0.23	-0.05

^aSolubility ratio calculated from S_{IPM}/S_{AQ} .

^b Δ log(SR) for compound and previous compound.

^cExperimental partition coefficient (C_{IPM}/C_{AQ}).

^d Δ log(PC) for compound and previous compound.

The direct aqueous solubility value for compound **18** agrees with the corresponding literature value included in Table 5-2. However, the value for compound **17** is lower than the literature value. The basis for this discrepancy is not clear.

In Table 5-3, the solubility ratios (SR) and experimentally determined partition coefficients (PC) are compared for the 3-acyl series. The $\log(PC) - \log(SR)$ values indicate that the partition coefficients are generally larger than the corresponding solubility ratios. As discussed in Chapter 2, this is common for polar solutes ($\log[PC] < 0$) that self-associate in the organic phase. The small negative $\log(PC) - \log(SR)$ value for compound **20** also fits the expected trend, but the large positive value for 3-butyryl-5FU (**19**) remains unexplained.

This deviation from the expected trend can also be seen in the hydrophobicity parameter (π) data. From the experimental partition coefficients, the $\Delta\log(PC)$ value is 0.96 for the comparison between compounds **18** and **19** and 0.23 for the comparison between compounds **19** and **20**, whereas the expected average π value is around 0.60. On the other hand, the $\Delta\log(PC)$ value for the comparison between compounds **18** and **20** is 0.60 per methylene unit. This suggests that the butyryl derivative in this series exhibits unusual solubility and partitioning properties compared to other members of the series.

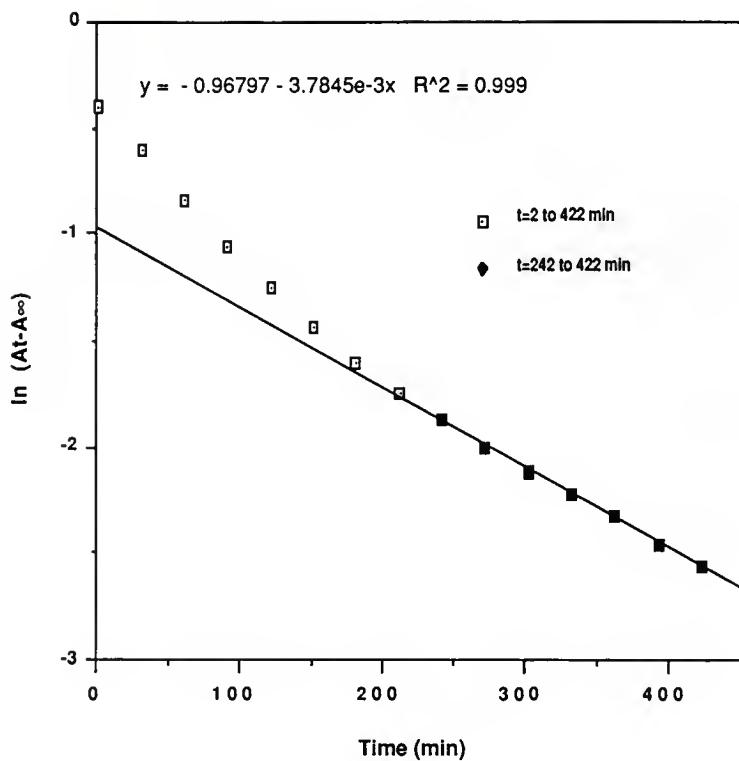


Figure 5-3. Plot of $\ln(A_t - A_{\infty})$ versus time (min) for hydrolysis of 3-acetyl-5FU in 0.05 M phosphate buffer (pH=7.1, I=0.12) at 32 °C (n=3).

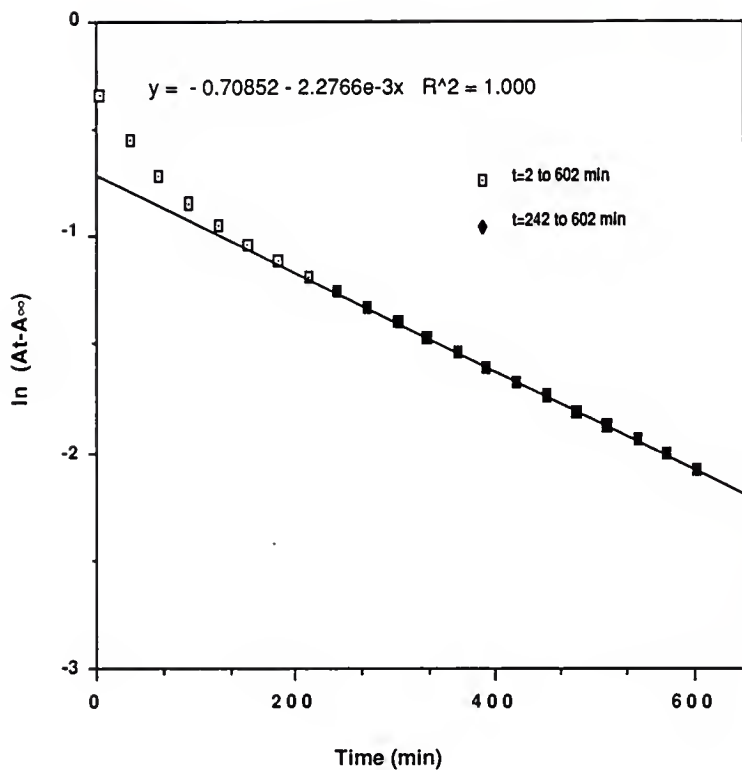


Figure 5-4. Plot of $\ln(A_t - A_\infty)$ versus time (min) for hydrolysis of 3-propionyl-5FU in 0.05 M phosphate buffer (pH=7.1, I=0.12) at 32 °C (n=2).

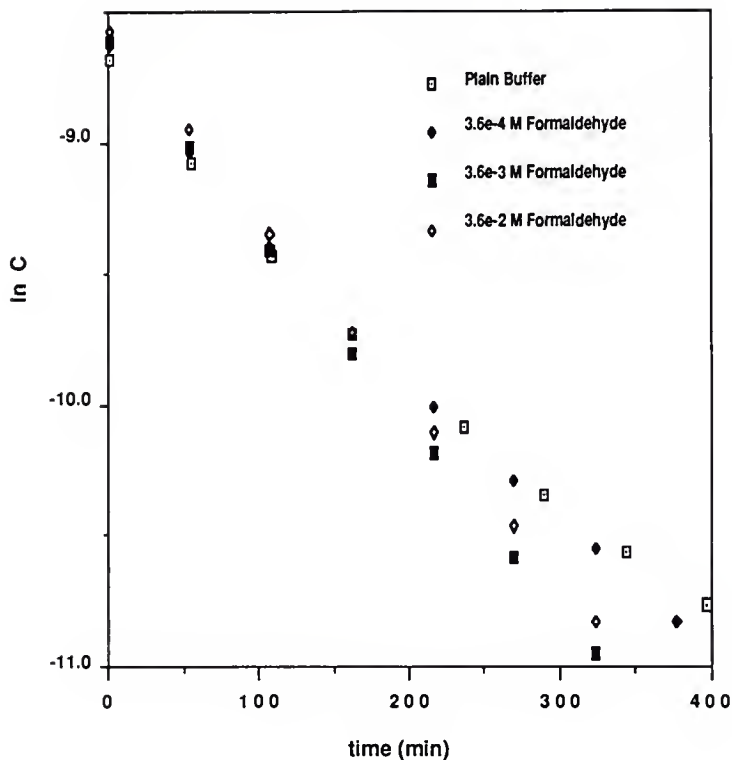


Figure 5-5. Plots of $\ln(C)$ versus time (min) for hydrolysis of 3-acetyl-5FU in 0.05 M phosphate buffer (pH=7.1, $I=0.12$) with ($n=2$) and without ($n=3$) formaldehyde at 32 °C.

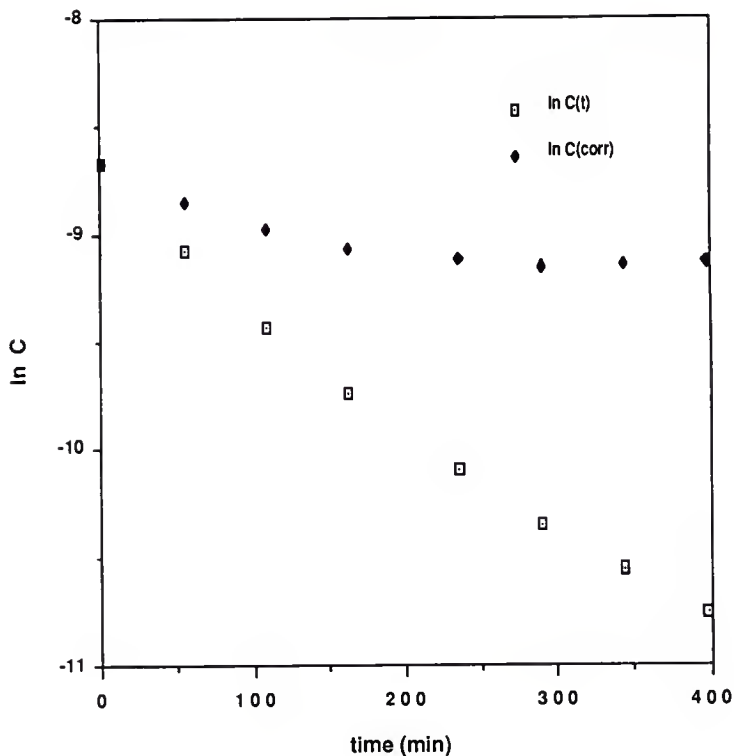


Figure 5-6. Plots of $\ln(C)$ versus time (min) for hydrolysis of 3-acetyl-5FU in 0.05 M phosphate buffer (pH=7.1, $I=0.12$) at 32 °C using actual concentration (C_t) and concentration corrected for secondary degradation (C_{corr}).

Table 5-4. Pseudo-first-order rate constants (k) and half-lives ($t_{1/2}$) for hydrolysis of 3-acyl derivatives in 0.05 M phosphate buffer (pH=7.1, $I=0.12$) with and without formaldehyde at 32 °C.

Compound (Assay)	Phase	Formaldehyde (M)	$k(\pm SD)^a$ (min^{-1})	$t_{1/2}$ (min)
17 (UV)	initial	0	$7.01 \times 10^{-3} (0.10 \times 10^{-3})$	99
17 (UV)	terminal	0	$3.91 \times 10^{-3} (0.23 \times 10^{-3})$	177
18 (UV)	initial	0	$6.20 \times 10^{-3} (0.14 \times 10^{-3})$	112
18 (UV)	terminal	0	$2.31 \times 10^{-3} (0.01 \times 10^{-3})$	300
17 (HPLC)	initial	0	$6.83 \times 10^{-3} (0.31 \times 10^{-3})^b$	101
17 (HPLC)	terminal	0	$3.92 \times 10^{-3} (0.32 \times 10^{-3})$	177
17 (HPLC)	initial	3.6×10^{-4}	$7.20 \times 10^{-3} (0.17 \times 10^{-3})$	96
17 (HPLC)	terminal	3.6×10^{-4}	$5.07 \times 10^{-3} (0.08 \times 10^{-3})$	137
17 (HPLC)	all	3.6×10^{-3}	$7.19 \times 10^{-3} (0.14 \times 10^{-3})$	96
17 (HPLC)	all	3.6×10^{-2}	$7.00 \times 10^{-3} (0.28 \times 10^{-3})$	99

^aMean $\pm$ standard deviation for $n=2-3$ values (see Figures).

^bCorrelation coefficient <0.999 (≈ 0.998).

Table 5-5. Reaction products formed during hydrolysis of 3-acetyl-5FU in 0.05 M phosphate buffer (pH=7.1, I=0.12) with formaldehyde at 32 °C.

Reactant (M)		Product (%)		
3-Acetyl-5FU	Formaldehyde	5FU	1-AOM-5FU ^a	3-AOM-5FU ^b
1.8x10 ⁻⁴	3.6x10 ⁻⁴	91	4.7	4.9
1.8x10 ⁻⁴	3.6x10 ⁻³	65	18	17
1.8x10 ⁻⁴	3.6x10 ⁻²	57	22	21

^a1-Acetyloxymethyl-5FU.

^b3-Acetyloxymethyl-5FU.

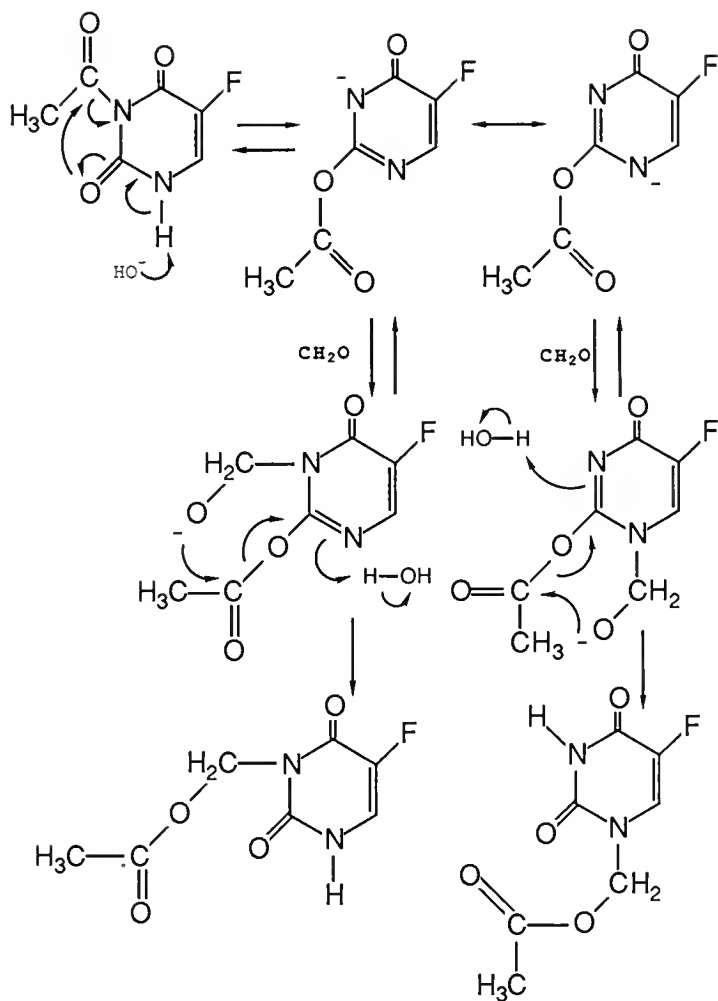


Figure 5-7. Possible scheme for reaction of 3-acetyl-5FU with formaldehyde to form 1-acetyloxymethyl-5FU and 3-acetyloxymethyl-5FU.

Hydrolysis Kinetics

Hydrolysis of 3-acetyl-5FU (**17**) to 5FU was followed by HPLC in 0.05 M phosphate buffer (pH=7.1, I=0.12) at 32 °C with and without formaldehyde and the hydrolyses of compounds **17** and **18** (3-propionyl-5FU) were followed by UV spectroscopy in the buffer alone. Disappearance of compounds **17** and **18** in the UV studies, indicated by $\ln(A_t - A_\infty)$, is plotted versus time (min) in Figure 5-3 and Figure 5-4 respectively. The plots appear to be biexponential with a short initial linear phase followed by curvature to an extended linear terminal phase.

In Figure 5-5, disappearance of compound **17** as $\ln(C)$ from the HPLC studies is plotted versus time (min). The same biexponential behavior is apparent for plain buffer and to a lesser extent for the lowest formaldehyde concentration. However, the plots for the two higher formaldehyde concentrations are linear throughout the course of the hydrolysis reactions.

Biexponential plots of this type suggest that the 3-acyl derivatives undergo rapid reversible reactions (initial phase) followed by slower hydrolysis reactions in which one or more of the compounds in equilibrium are converted to 5FU (terminal phase). The pseudo-first-order plots, obtained when formaldehyde is added to the buffer, indicate that formaldehyde must be trapping the product or products of the initial reaction thereby preventing the reverse reaction.¹¹⁴⁻⁵

In Figure 5-6, the HPLC plot for compound **17** is shown along with a corrected concentration plot from the equation:¹¹⁴⁻⁵

$$\ln(C_{\text{corr}}) = \ln(C_t) + kt \quad (7)$$

where C_{corr} is the concentration of compound **17** after correcting for terminal phase hydrolysis and k is the observed rate constant for the linear terminal phase. The flattened appearance of the $\ln(C_{\text{corr}})$ versus time plot supports the assertion that a reversible reaction is involved as the initial step in the hydrolysis of compound **17**.¹¹⁴⁻⁵

In Table 5-4, rate constants (k) and half-lives ($t_{1/2}$) from the preceding plots are presented. The UV and HPLC data for compound **17** are consistent, and the initial-phase rate constants are essentially the same regardless of the amount of formaldehyde added to the buffer. The linearity of the plots with the higher formaldehyde concentrations is indicated by a single rate constant.

Evidence that formaldehyde is trapping an intermediate is presented in Table 5-5. Although an intermediate was never observed during the HPLC studies, two products were identified that suggest an O^2 -acylated intermediate. The formation of 1-acetyloxymethyl-5FU and 3-acetyloxymethyl-5FU in equal concentrations was confirmed by comparison with authentic samples.¹ Identical retention times from the HPLC chromatograms were observed for the authentic samples and the

¹ Authentic samples were provided by Dr. Hans Bundgaard of the Royal Danish School of Pharmacy, Copenhagen, Denmark.

reaction products from the hydrolysis studies, and ^1H NMR spectra obtained from a scaled-up hydrolysis reaction were consistent with the assigned structures based on the positions of the $\text{C}^6\text{-H}$ and $\text{N-CH}_2\text{-O}$ absorptions.²³ In addition, 1-acetyloxymethyl-5FU was isolated from the reaction mixture, and its melting point ($124\text{-}5^\circ\text{C}$) was consistent with the literature-reported value ($122\text{-}3^\circ\text{C}$).²²

A possible scheme for formation of 1- and 3-acetyloxymethyl-5FU is presented in Figure 5-7. The even distribution of the two products at all formaldehyde concentrations (Table 5-5) suggests an intramolecular reaction with an $\text{O}^2\text{-acyl}$ intermediate.

Skin Penetration

Skin penetration data from the diffusion cells are plotted as cumulative amount of total 5FU species that diffused (μmol) versus time (h). In Figure 5-8, results for 3-acetyl-5FU (**17**) and 3-propionyl-5FU (**18**) are compared to 5FU itself. In Figure 5-9, results for 3-butyryl-5FU (**19**) and 3-valeryl-5FU (**20**) are compared to 5FU. Error bars correspond to the standard deviation from the mean for $n=3$ values.

Values for cumulative amount of total 5FU species that diffused and skin accumulation of total 5FU species were obtained by UV analysis of the receptor phases. Since the UV spectra showed lower $\text{UV}_{\text{max}}/\text{UV}_{\text{min}}$ ratios than expected for 5FU

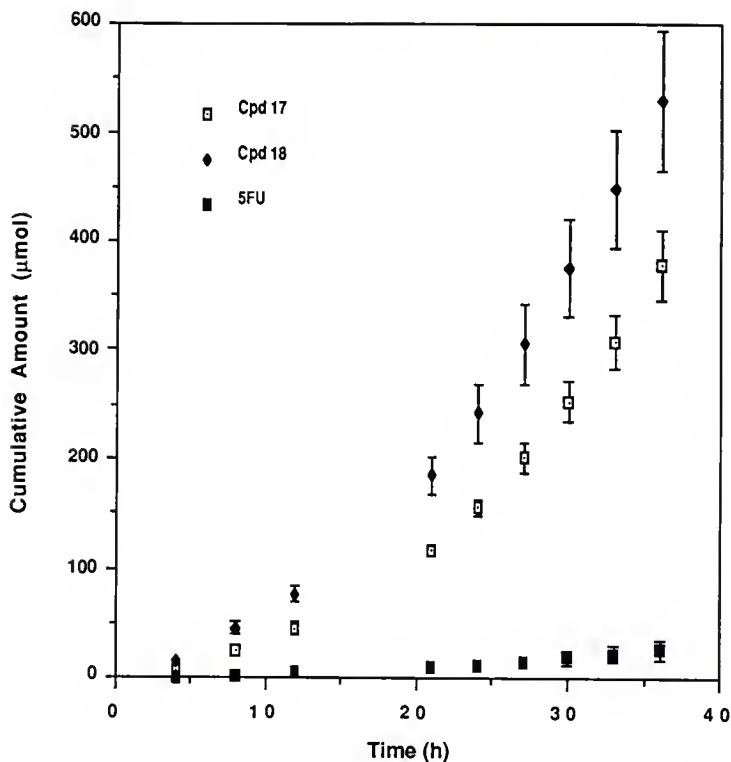


Figure 5-8. Plots of cumulative amount of total 5FU species that diffused (μmol) versus time (h) for compounds 17, 18, and 5FU.

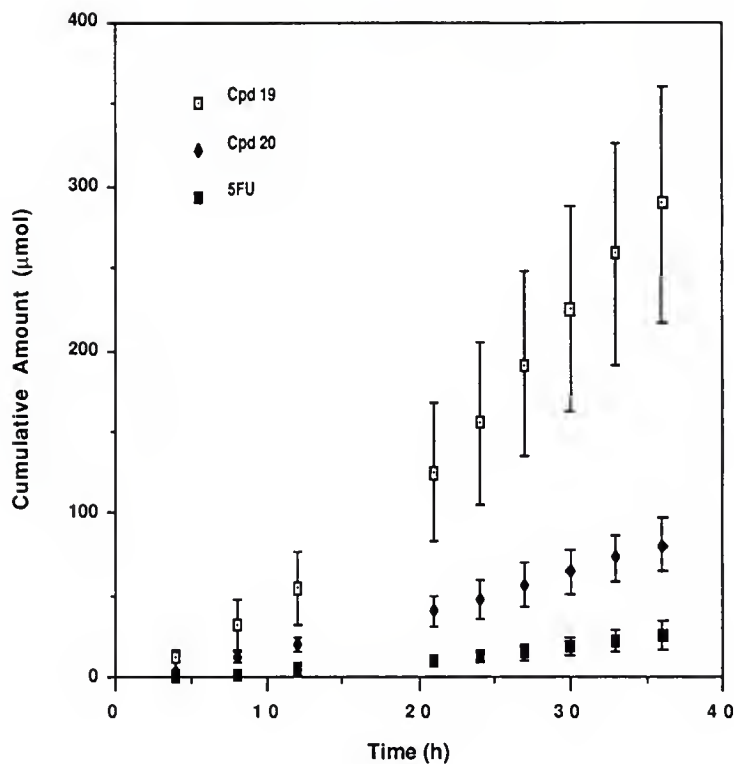


Figure 5-9. Plots of cumulative amount of total 5FU species that diffused (μmol) versus time (h) for compounds 19, 20, and 5FU.

Table 5-6. Fluxes (J), lag times (t_L), and skin accumulation (SA) values for 3-acyl derivatives.

Compound	J($\pm$ SD) ^a ($\mu\text{mol}/\text{cm}^2/\text{h}$)	Prodrug S-S ^b (11 h) ^c (%)	t_L (h)	SA($\pm$ SD) ^a (μmol)
5FU	0.24 (0.09)	-	13	3.7 (0.9)
17	4.4 (0.5)	24 (19)	19	16 (3)
18	5.2 (0.7)	49 (43)	15	15 (2)
19	2.2 (0.3)	62 (52)	9.8	7.1 (1.6)
20	0.55 (0.07)	-	6.2	3.3 (1.1)

^aMean $\pm$ standard deviation for n=3 values.

^bPercent of total 5FU as intact prodrug during "steady-state" phase in separate experiment (n=1).

^cPercent of total 5FU as intact prodrug from 11 h sample in separate experiment (n=1).

Table 5-7. Second application fluxes (J) and lag times (t_L) for 3-acyl derivatives.

Compound	$J^a (\pm SD)^b$ ($\mu\text{mol}/\text{cm}^2/\text{h}$)	t_L (h)
5FU	1.2 (0.2)	1.2
17	1.6 (0.1)	0.5
18	1.8 (0.2)	1.2
19	1.1 (0.1)	1.0
20	1.1 (0.2)	1.3

^aFlux of 0.4 M theopylline from propylene glycol.

^bMean $\pm$ standard deviation for n=3 values.

alone and hydrolysis studies showed that products other than 5FU were formed during hydrolysis of the 3-acyl derivatives in formaldehyde buffer, it was necessary to compare the molar absorptivity (ϵ) at 266 nm for 5FU with ϵ values at 266 nm for hydrolyzed 3-acyl and 1,3-bis-acyl derivatives in solutions containing receptor phase. Solutions containing known concentrations of each of the 3-acyl and 1,3-bis-acyl derivatives were incubated for 24 hours at 32 °C at which time HPLC analysis showed that only 5FU and the two formaldehyde reaction products were present. Subsequent analysis by UV spectroscopy showed that the multiple component solutions had ϵ values that were within $\pm 5\%$ of the ϵ value for 5FU alone at 266 nm. This verified that the UV assay for determining cumulative amount of total 5FU species that diffused and skin accumulation of total 5FU species is valid.

Fluxes (J), lag times (t_L), and skin accumulation (SA) values for each compound are reported in Table 5-6. Lag time refers to the intersection of the linear, or "steady-state," region of each graph with the time (x) axis, and it is the time required for establishing a uniform concentration gradient within the skin.⁶⁵ The percentages of total 5FU present as intact prodrugs in the receptor phases are also reported in Table 5-6. These values were calculated from samples taken during the "steady-state" phase and from an earlier sample (11 h) in a separate experiment using HPLC analysis ($n=1$).

All of the 3-acyl derivatives improved the skin penetration of 5FU. Increases in flux ranged from more than two times (3-valeryl-5FU [20]) to more than 20 times (3-propionyl-5FU [18]) the flux of 5FU itself.

As was the case with the 1-alkyloxycarbonyl derivatives, large amounts of intact 3-acyl prodrugs were detected in the receptor phases. Unlike the 1-alkyloxycarbonyl derivatives, however, the differences between the "steady-state" percentages and the percentages calculated from samples taken prior to "steady-state" are small. This suggests that hydrolytic enzymes play a relatively minor role in the hydrolysis of the 3-acyl derivatives when compared with the 1-alkyloxycarbonyl derivatives. This is consistent with the known hydrolytic behavior of the 3-acyl series in human plasma²⁶ and is probably due to the steric and electronic effects described earlier. It is important to note that the intact prodrug percentages reported in Table 5-6 represent the lower limits of prodrug content. Formaldehyde reaction products (1- and 3-acyloxymethyl derivatives) were also present in the receptor phases at the time of analysis which indicates that more than the observed amounts of 3-acyl derivatives had actually diffused through the skin intact.

Skin accumulation values are highest for compounds 17 and 18 and are over four times higher than the value for 5FU. Overall, skin accumulation is much lower from the 3-acyl derivatives than the 1-acyl derivatives which supports the

earlier assertion that rapid hydrolysis of 5FU prodrugs leads to higher skin levels of 5FU.

Second application fluxes and lag times are reported in Table 5-7. Skin penetration by theophylline from propylene glycol, the standard drug-vehicle combination, after treatment with the 3-acyl derivatives is similar to that following treatment with 5FU.

The stability of the prodrugs in the IPM formulations was assessed by ^1H NMR analysis of the donor phases. After a minimum of five days from the time the suspensions were prepared until their ^1H NMR spectra were recorded, including at least twelve hours during which the formulations were in contact with the skins, the 3-acyl derivatives were found to be intact with no evidence of 5FU formation.

Summary

The 3-acyl derivatives of 5FU exhibited increased lipid solubilities when compared to 5FU. Aqueous solubility reached a maximum for 3-propionyl-5FU (**18**) and then decreased with increasing chain length. Skin penetration and skin accumulation were also maximized for compound **18** suggesting that both lipid and aqueous solubilities are important for predicting transdermal and dermal delivery of these 5FU prodrugs. The presence of high percentages of prodrugs in the receptor phases, indicating insufficient release of the parent drugs in the hairless mouse skin model, may limit the

potential of this series of prodrugs at least for dermal delivery purposes. The partitioning-based method for determining aqueous solubility was effective for determining the relative solubilities in the 3-acyl series, but overall, the agreement with the direct method for determining aqueous solubility was not as good as it was for the 1-alkyloxy-carbonyl series. An O²-acyl intermediate was proposed to explain the thermal decomposition and rearrangement of the 3-acyl derivatives and the unusual hydrolytic behavior of the series including the formation of acyloxymethyl derivatives of 5FU.

CHAPTER 6 SUMMARY AND CONCLUSIONS

Fluorouracil (5FU) is a commercially available antineoplastic agent that has been used topically for treatment of actinic keratoses, superficial basal cell carcinomas, psoriasis, and other precancerous conditions, malignant and benign tumors, and dermatoses. As a typical polar, heterocyclic compound, however, 5FU has poor solubility and skin penetration properties, and topical treatment with 5FU is often ineffective.

In an attempt to improve the topical delivery of 5FU, four homologous series of bioreversible derivatives (prodrugs) of 5FU were synthesized and characterized, and their ability to penetrate through and accumulate in the skin was evaluated. The four series included six 1-alkyloxy-carbonyl, six 1-acyl, four 1,3-bis-acyl, and four 3-acyl derivatives.

Solubilities and melting points for the four series of prodrugs showed the expected trends. Melting points generally decreased and lipid solubilities generally increased with increasing chain length, and in fact, lipid solubilities were a minimum of 40 times greater for the derivatives than for 5FU. Aqueous solubilities were maximized for the first or second member of each series and then decreased with increasing chain length. Five

derivatives had aqueous solubilities greater than 5FU even though an hydrogen-bonding N-H group was masked in each. The partitioning-based method for determining aqueous solubilities was shown to give reliable values for relative solubility in a homologous series, and overall, the values correlated well with the conventionally determined aqueous solubilities. It is noteworthy that this is the first known successful attempt to obtain aqueous solubilities for such chemically unstable prodrugs.

Hydrolysis of the 1-alkyloxycarbonyl, 1-acyl, and 1,3-bis-acyl (N^1 -acyl group only) derivatives followed pseudo-first-order kinetics in 0.05 M phosphate buffer (pH=7.1, $I=0.12$) with and without formaldehyde which was used as a preservative in the diffusion cell experiments. Hydrolysis was faster in the presence of formaldehyde which apparently functions as a general base catalyst. The N^1 -acyl groups were much more rapidly hydrolyzed than the N^3 -acyl groups even though the N^3 -anion is a better leaving group than the N^1 -anion. The X-ray crystal structure of 1,3-bis-acetyl-5FU showed that the N^3 -acetyl group was oriented perpendicular to the plane of the 5FU ring, and the carbonyl carbon was sterically and electronically hindered from attack by hydroxide ion. It was suggested that the unusual orientation of the N^3 -acyl group caused the slower than expected hydrolysis rates for the 3-acyl derivatives compared to the 1-acyl derivatives.

Hydrolysis of the 3-acyl derivatives was biexponential. A reversible reaction involving an O-acyl intermediate was proposed for the initial phase followed by terminal phase hydrolysis of the 3-acyl derivative, an O-acyl intermediate, or both to 5FU. Formation of nearly equal amounts of 1- and 3-acetyloxymethyl-5FU during hydrolysis of 3-acetyl-5FU in buffer with formaldehyde suggested that the intermediate may be the O²-acetyl derivative.

An O²-acyl intermediate was also proposed to explain the thermal decomposition and rearrangement of the 3-acyl derivatives to form N¹-acylated compounds. Ketene formation was proposed to account for the formation of 5FU from the 3-acyl derivatives (and possibly the 1-acyl derivatives) during their thermal decomposition.

Skin penetration was 1.2 to nearly 40 times greater for the prodrugs than for 5FU itself. The highest flux was recorded for 1-acetyl-5FU, whereas 3-propionyl-5FU, 1-ethyloxycarbonyl-5FU, and 1,3-bis-acetyl-5FU exhibited the highest rates of delivery of 5FU for their respective series. Without exception, the highest fluxes were obtained for the most aqueous soluble derivative in each series. The skin penetration results demonstrate the importance of biphasic solubility for achieving optimal transdermal delivery of 5FU from a homologous series of prodrugs.

Intact prodrug content in the receptor phases constituted over 40% of total 5FU species that had diffused for the 1-alkyloxycarbonyl and the 3-acyl derivatives with

the exception of 3-acetyl-5FU which was much lower. Since the 1,3-bis-acyl derivatives hydrolyzed to give the corresponding 3-acyl derivatives almost immediately, they should behave in a similar manner to the 3-acyl derivatives. Testing of the 3-acyl, 1,3-bis-acyl, and 1-alkyloxycarbonyl series in other skin models should be carried out before they are dismissed as too stable for use as prodrugs for dermal as opposed to transdermal delivery.

Skin accumulation of total 5FU species was highest for the 1-acyl derivatives particularly for 1-acetyl-5FU and 1-propionyl-5FU. These two prodrugs had skin accumulation values more than 18 times the value for 5FU and nearly four times more than the next best derivative. It was suggested that rapid hydrolysis of the 1-acyl derivatives upon partitioning into the skin effectively "locks in" large amounts of 5FU since it is the highly polar 5FU molecule that must diffuse through the remaining lipid regions of the skin.

Second application studies using the standard drug-vehicle combination showed that skin damage was at most one and one-half times greater for the prodrugs than for 5FU, a small increase when compared to the much greater improvement in skin penetration and skin accumulation that was achieved.

The stability of the prodrugs in the IPM formulations was documented and no decomposition was observed even after a twelve-hour period during which the formulations were in contact with the skins. This shows that even chemically

unstable derivatives can potentially be formulated for topical delivery.

Overall, 1-acetyl-5FU was the most effective prodrug for delivering 5FU through the skin (transdermal) and retaining 5FU in the skin (dermal). The combination of high flux, rapid hydrolysis, high skin accumulation, and stability in formulation makes 1-acetyl-5FU the best candidate for improving the dermal or transdermal delivery of 5FU.

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BIOGRAPHICAL SKETCH

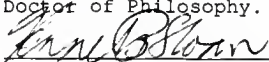
Howard D. Beall was born July 6, 1954, in Salt Lake City, Utah. The Beall family returned to Montana before he was two years old, and he spent his school years in several Montana cities.

He graduated with honors from the University of Montana in 1977 with a Bachelor of Science degree in pharmacy. He practiced pharmacy in various settings in Montana and Oregon before moving to Florida and earning a Master of Science degree in pharmacy from the University of Florida in 1983. He entered the doctoral program in the Department of Medicinal Chemistry at the University of Florida in 1987.

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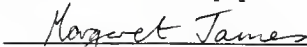
He married Donna Goyette in 1984, and they have a son, Michael, born in 1989, and a daughter, Allison, born in 1991.

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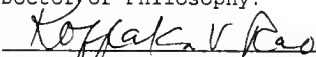
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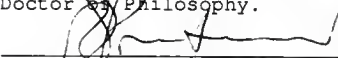
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This dissertation was submitted to the Graduate Faculty of the College of Pharmacy and to the Graduate School and was accepted as partial fulfillment of the requirements for the degree of Doctor of Philosophy.

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